9-15-2016

Phylogenetics of extant and fossil Pinaceae: Methods for increasing topological stability

David S. Gernandt
Universidad Nacional Autónoma de México

Garth Holman
University of Maine

Christopher Campbell
University of Maine

Matthew Parks
Oregon State University

Sarah Mathews
Commonwealth Scientific and Industrial Research Organization

See next page for additional authors

Recommended Citation

Gernandt, D., Holman, G., Campbell, C., Parks, M., Mathews, S., Raubeson, L., Liston, A., Stockey, R., & Rothwell, G. (2016). Phylogenetics of extant and fossil Pinaceae: Methods for increasing topological stability. *Botany, 94* (9), 863-884. https://doi.org/10.1139/cjb-2016-0064

NRC Research Press

ARTICLE

Phylogenetics of extant and fossil Pinaceae: methods for increasing topological stability[1]

David S. Gernandt, Garth Holman, Christopher Campbell, Matthew Parks, Sarah Mathews, Linda A. Raubeson, Aaron Liston, Ruth A. Stockey, and Gar W. Rothwell

Abstract: Relationships of living and fossil Pinaceae were inferred using parsimony and Bayesian inference of morphological characters and plastid and nuclear DNA sequences. When considering extant taxa only, adding molecular to morphological characters resulted in markedly increased resolution and branch support compared with analysis of morphology alone. Including 45 fossil taxa resulted in drastically decreased resolution in morphology-based consensus trees. We evaluated the effect on branch support and resolution of including DNA sequences, deleting fossils lacking information for cone scale apices and seeds, using reduced consensus methods, and using implied weighting, and found that the greatest improvements were found by including DNA sequences and using implied weighting. The tree topologies from parsimony and Bayesian inference confirm previous findings that the fossil genus *Pseudoaraucaria* and a few species of *Pityostrobus* from the Lower Cretaceous are related to abietoid genera, and that other species of *Pityostrobus* are pinoid and closely related to *Pinus*. Focusing phylogenetic analyses on the most complete fossil cones, specifically those that are anatomically preserved and include both cone scale apices and seeds, and taking into account homoplasy, resulted in the clearest hypotheses for the timing and sequence of diversification in the family.

Key words: fossil, morphology, phylogenetics, Pinaceae, *PHYP*.

Résumé : Les relations qui existent entre les Pinaceae vivants et fossiles ont été déduites par la parcimonie et l'inférence Bayésienne des traits morphologiques et des séquences d'ADN plastidique et nucléaire. En considérant les taxons encore existants seulement, l'ajout de traits moléculaires aux traits morphologiques résultait en un accroissement marqué de la résolution et du support des branches comparativement à l'analyse de la morphologie seule. L'inclusion de 45 taxons fossiles résultait en une résolution fortement diminuée dans les arbres consensuels basés sur la morphologie. Les auteurs ont évalué l'effet d'inclure les séquences d'ADN sur le support des branches et la résolution, en supprimant les fossiles pour lesquels l'information sur les apex des écailles des cônes et des graines était manquante, en utilisant des méthodes de consensus réduites, et en utilisant une pondération implicite, et ils ont trouvé que les plus grandes améliorations étaient obtenues en incluant les séquences d'ADN et en utilisant la pondération implicite. Les topologies des arbres à partir de la parcimonie et de l'inférence Bayésienne confirment les résultats précédents voulant que les fossiles du genre *Pseudoaraucaria* et quelques espèces de *Pityostrobus* du Crétacée inférieur soient apparentés au genre abiétoïde et que les autres espèces de *Pityostrobus* soient pinoïdes et étroitement apparentées aux *Pinus*. En concentrant les analyses phylogénétiques sur les cônes fossiles les plus complets, spécifiquement ceux qui sont anatomiquement préservés et qui comprennent les apex des écailles des cônes et les graines, et en considérant l'homoplasie, on obtient les hypothèses les plus claires quant à la chronologie et la séquence de la diversification dans la famille. [Traduit par la Rédaction]

Mots-clés : fossile, morphologie, phylogénétique, Pinaceae, *PHYP*.

Received 2 March 2016. Accepted 18 June 2016.

D.S. Gernandt. Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, 04510, Mexico.
G. Holman and C. Campbell. School of Biology and Ecology, University of Maine, Orono, ME 04469, USA.
M. Parks,* A. Liston, and R.A. Stockey. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA.
S. Mathews. CSIRO National Research Collections Australia, Australian National Herbarium, Canberra, ACT 2601, Australia.
L.A. Raubeson. Department of Biological Sciences, Central Washington University, Ellensburg, WA 98926, USA.
G.W. Rothwell. Department of Environmental and Plant Biology, Ohio University, Athens, OH 45701, USA; Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA.
Corresponding author: David S. Gernandt (email: dgernandt@ib.unam.mx).
*Current address: Daniel F. and Ada L. Rice Plant Conservation Science Center, Chicago Botanic Gardens, Glencoe, IL 60022, USA.
[1]This Article is part of a Special Issue entitled "Mesozoic and Cenozoic Plant Evolution and Biotic Change," a collection of research inspired by, and honouring, Ruth A. Stockey.

Introduction

Simultaneous phylogenetic analysis of extant and extinct taxa permits richer hypotheses for the evolutionary history of groups. Including fossils or other extinct species is important for providing alternative hypotheses for phylogenetic relationships and of character polarization, but, when extinct taxa are known only from imperfectly preserved or fragmentary material lacking informative characters, the taxa can be difficult to place in phylogenies (Donoghue et al. 1989; Nixon 1996). Empirical and simulation studies have shown that adding fossils to phylogenetic datasets can result in decreased resolution when not enough characters are scored or when too many fossils are added with respect to extant taxa with more complete data (Wiens 2006; Wiens et al. 2010). Use of explicit, well-justified criteria for choosing which fossils to include in phylogenetic analyses facilitates the recovery of more accurate and robust phylogenetic hypotheses, thus providing a clearer picture of the evolution of taxa and characters in time and space. Choice of inference methods can also have an impact on phylogenetic accuracy. Parsimony with equal weights (maximum parsimony) is a widely accepted method, particularly for analysing morphological data, but downweighting homoplasious characters with parsimony or using model-based methods often gives more consistent results in simulation and empirical studies (Hillis et al. 1994; Goloboff et al. 2008*a*).

Extant Pinaceae comprises 11 genera and approximately 200 species of trees and a few shrubs that dominate many terrestrial vegetation types in the Northern Hemisphere, particularly boreal, temperate, and montane forests (Eckenwalder 2009; Farjon 2010). Much of our understanding of the early evolution and diversity of the family is based on isolated permineralized seed cones from the Cretaceous and Paleogene of Eurasia and North America (reviewed by Miller 1976; Klymiuk et al. 2012; Smith et al. 2016). These cones are structurally diverse and thought to include both representatives of modern genera and extinct lineages (Miller 1976). The fossil seed cones that do not correspond to living genera have been classified into one of four organ genera: *Pityostrobus* Nathorst emend. Dutt, a polyphyletic assemblage of about 29 described species; *Obirastrobus* Ohsawa, Nishida et Nishida, possibly a natural genus with two species; *Pseudoaraucaria* Fliche, a natural genus with six species; and *Eathiestrobus mackenziei* Rothwell, Mapes, Stockey et Hilton, described recently from a Late Jurassic seed cone from Scotland and possibly representing the oldest record for an extinct genus of Pinaceae (Rothwell et al. 2012). Early Cretaceous seed cones attributed to *Picea* A. Dietr. and *Pinus* L. arguably represent the oldest records for extant genera (Alvin 1960; Klymiuk and Stockey 2012; Ryberg et al. 2012). *Pinus arnoldii* Klymiuk, Stockey et Rothwell from the Eocene of British Columbia is the only extinct species of Pinaceae with a morphological concept based on a whole-plant reconstruction (Klymiuk et al. 2011). Fossils of Pinaceae are first recognized from the Late Jurassic to Early Cretaceous, and like living species are geographically restricted to the Northern Hemisphere (except one extant pine with an equatorial distribution), suggesting that the family evolved in Laurasia after its separation from Gondwana in the Early Jurassic.

Intergeneric phylogenetic relationships of extant Pinaceae are relatively well studied. Most analyses recover a pinoid (subfamily Pinoideae) clade comprising *Pinus*, *Picea*, *Cathaya* Chun & Kuang, *Larix* Mill., and *Pseudotsuga* Carrière, and an abietoid (subfamily Abietoideae sensu Frankis 1988) clade comprising *Abies* Mill., *Keteleeria* Carrière, *Nothotsuga* Hu ex C.N. Page, *Tsuga* Carrière, and *Pseudolarix* Gordon. Less consistently, *Cedrus* Mill. is the sister group to the previous five genera (Hart 1987; Price et al. 1987; Wang et al. 2000; Gernandt et al. 2008; Lin et al. 2010; Holman 2014; Lu et al. 2014). Recovery of two principal Pinaceae clades agree with Van Tieghem's (1891) classification based on morphological and anatomical evidence, but the abietoid genera are occasionally recovered as paraphyletic because of the position of *Cedrus*, which sometimes is recovered sister to Pinaceae, or more rarely to the pinoid clade (Hart 1987; Wang et al. 2000; Gernandt et al. 2008). Paraphyletic abietoid topologies suggest that at least some abietoid lineages diverged prior to the pinoid clade. Most of the oldest Pinaceae fossils assigned to living genera are pinoid (*Picea* and *Pinus*), but wood of an abietoid genus has also been reported (*Cedrus*; Blokhina and Afonin 2007). Furthermore, some extinct taxa known from the Lower Cretaceous, such as the genus *Pseudoaraucaria*, have abietoid features such as their seed scale morphology and the presence of resin cavities in their seed integument (Alvin 1988). The phylogenetic relationships among *Cathaya*, *Pinus*, and *Picea* are also uncertain.

Several studies have addressed the relationship of fossil and extant Pinaceae. Alvin (1988) reported a distance analysis of 22 seed cone characters and 11 taxa that recovered the fossil genus *Pseudoaraucaria* as sister to extant abietoid genera, but no other fossil taxa or outgroups were included at that time. Smith and Stockey (2001, 2002) presented cladistic analyses of an expanded and recoded data set of 33 morphological and anatomical characters and 48 taxa (70% of which were fossils) that recovered *Pseudoaraucaria* (with six species) as monophyletic and sister to *Abies*; the fossil genus *Obirastrobus* (two species) was recovered as monophyletic (only in a majority-rule consensus tree), and the fossil genus *Pityostrobus* (25 species) was polyphyletic; the positions of *Obirastrobus* and *Pityostrobus* were poorly resolved. In a cladistic analysis of 50 taxa and 53 seed cone characters, including the extant genus *Pinus* represented by two extant and one fossil species rather than a single generic terminal, *Pseudoaraucaria* was recovered as sister to *Abies*, and more than 13 species of *Pityostrobus* as close relatives of *Pinus*, with very low branch support (Gernandt et al. 2011). Pol and Escapa (2009) used the Smith and Stockey

(2002) matrix to illustrate a method for iteratively identifying and pruning taxa with an unstable position among optimal trees to generate a reduced consensus tree. This unfortunately required pruning 12 taxa from the trees, including four of the family's 11 extant genera (Supplementary data, Fig. S1[2]). Better resolved, but similarly non-robust results have been reported for combined analyses with plastid DNA sequences (Gernandt et al. 2008; Ryberg et al. 2012). The topological instability observed in phylogenetic analyses of extinct and living Pinaceae could be attributed to character conflict, insufficient characters, or both.

This study addresses the phylogenetic relationships among modern and fossil Pinaceae using an expanded morphological data set that is analysed separately and combined with molecular data consisting of a concatenated alignment of plastome exons and nuclear *PHYP* sequences. Ongoing study of living and fossil species to clarify morphological character concepts and coding decisions and an increase in the number of scored characters is expected to steadily increase phylogenetic accuracy for this group (Nixon and Davis 1991). Our objectives are to (*i*) infer phylogenetic relationships among extant species and previously analysed pinaceous fossils using separate and combined analysis of molecular and morphological data, (*ii*) compare topological stability and branch support across separate and combined analyses and under different character weights, and (*iii*) identify a reduced set of fossil taxa that are well-preserved and capture the early phylogenetic diversity of the family. This total evidence approach yields the most robust hypotheses for the phylogeny of Pinaceae thus far achieved.

Materials and methods

Taxon and character sampling

Previous phylogenetic studies of Pinaceae either treated all genera as terminal taxa (e.g., Hart 1987; Alvin 1988), or used a combination of species (mainly fossils) and genera (mainly extant taxa) as terminals (e.g., Smith and Stockey 2001, 2002; Gernandt et al. 2008, 2011; Smith et al. 2016). In contrast, we chose 67 species-level terminals to represent Pinaceae. These included 23 extant species representing all 11 living genera, one fossil species for which a whole organism reconstruction is available (*Pinus arnoldii*), and 39 species known only from permineralized ovulate cones (Supplementary data, Tables S1 and S2). The most species rich genera, *Pinus*, *Abies*, *Picea*, and *Larix*, included eight, three, three, and two (extant) species, respectively. Eleven outgroups were included: six extant conifers representing as many families, one species described solely from seed cones (*Pararaucaria patagonica* Wieland emend. Escapa, Rothwell, Stockey et Cúneo) and classified in the extinct family Cheirolepidiaceae, four fossil coniferophytes for which whole organism reconstructions are available (Cordaitaceae, Emporiaceae, and Bartheliaceae), and one other extant gymnosperm (*Ginkgo biloba* L.).

Parauracaria patagonica, described from the Cerro Cuadrado Petrified Forest in Argentina, was recently assigned to Cheirolepidiaceae (Escapa et al. 2012). Its seed cone shares characters with Araucariaceae, Pinaceae, and taxodiacious Cupressaceae (Stockey 1977). It is similar to Pinaceae in possessing seed cones with helically arranged bract–scale complexes that are fused only at the base, and in some species having two inverted ovules. It differs in lacking resin canals, possessing lobed scale apices, lacking seed wings, and in most species having only one seed per scale (Escapa et al. 2012, 2013). Fossils of Cheirolepidiaceae occur in Triassic through Cretaceous deposits worldwide (Escapa et al. 2012). For the morphological matrix, we chose representatives of the outgroup families Taxaceae and Podocarpaceae with recognizable cones (*Cephalotaxus* Siebold & Zucc. ex Endl., and *Phyllocladus* Rich. ex Mirb., respectively). There were three cases in the outgroup of terminal mismatch for which the morphological and molecular matrices used different taxa: *Araucaria heterophylla* (Salisb.) Franco was used in the morphology matrix, whereas *Araucaria columnaris* (G. Forst) Hook. was used for nuclear phytochrome P (*PHYP*) and plastome sequences; *Phyllocladus hypophyllus* Hook.f. was used for the morphology matrix, whereas *Podocarpus macrophyllus* (Thunb.) Sweet was used for *PHYP* and plastome sequences; and *Cephalotaxus harringtonii* (Knight ex J. Forbes) K. Koch was used for morphology and plastome sequences, whereas *Cephalotaxus sinensis* (Rehder & E.H. Wilson) H.L. Li was used for the *PHYP* sequences. There was one additional case of terminal mismatch in Pinaceae: *Pinus pinaster* Aiton was used in the morphology matrix and for plastome sequences, whereas *Pinus pinea* L. was used for *PHYP*. In these cases, data sources for different species were fused for the combined analyses.

A matrix of morphological and other structural characters was scored in Mesquite version 2.75 (Maddison and Maddison 2010). Character definitions and scorings were from previously published phylogenetic analyses (Hart 1987; Farjon 1990; Nixon et al. 1994; Rothwell and Serbet 1994; Smith and Stockey 2001, 2002; Gernandt et al. 2008, 2011; Ryberg et al. 2012; Smith et al. 2016), literature review (Radais 1894; Jeffrey 1905; Bailey 1909; Gerry 1910; Chamberlain 1935; Sacher 1954; Greguss 1955; Behnke 1974; Huerta Crespo 1976; Hu and Wang 1984; Baas et al. 1986; Frankis 1988; Lin et al. 1995; Wu and Hu 1997; Ickert-Bond 2000; Whang et al. 2001, 2004; Esteban and De Palacios 2009; Powell 2009; Eckenwalder 2009; Farjon 2010), and new observations. We retained characters with missing data for some taxa, following the argument that this can increase phylogenetic accuracy (Wiens 2006).

[2]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjb-2016-0064.

Character definitions and references to previous studies are indicated in Appendix A. We used contingent coding (Forey and Kitching 2000) to distinguish between presence or absence characters ("neomorphic" sensu Sereno 2007) and characters describing alternate, independent, and mutually exclusive states ("transformational" sensu Sereno 2007). Thus, when a structure was determined to be absent in a taxon, any dependent transformational characters were scored as inapplicable. A total of 158 characters were included: branching and growth (8), vegetative shoot anatomy (24), root (2), foliar (27), pollen cones (13), seed cones (80), and biochemistry (4). The seed cone was further subdivided into cone morphology (26), axis anatomy (13), bract anatomy (6), scale anatomy (10), fertilization and embryology (8), and seed (17). All characters were informative for the complete set of taxa (extant and fossil), but some were uninformative in analyses with subsets of taxa. Thirty-two characters were multistate and the rest were binary. Fifteen multistate characters were treated as ordered. These represented counts, measurements, or qualitative states that could be considered as a transformational series. The average number of scored characters was 124 for extant Pinaceae (range 98–148, or 3.9–39.0% missing), 114 for extant outgroups (range 98–134, or 10.1–30.9% missing), and 55 for fossils (range 40–104, or 33.8%–74.5% missing).

DNA was extracted using a modified CTAB method (Doyle and Doyle 1987) or a Wizard Genomic DNA purification kit (Promega, Madison, Wisconsin, USA). Sequence data sets were assembled for the first exon of the nuclear *PHYP* gene and for 73 plastid genes. New sequences were determined for Pinaceae *PHYP* using Sanger sequencing. Previously published (García-Gil et al. 2003; Pyhäjärvi et al. 2007) and newly designed primers used for sequencing *PHYP* are given in the Supplementary data, Fig. S2. Sequences for the plastid genes were determined using Illumina sequencing as described in Cronn et al. (2008), Parks et al. (2009, 2012), and Holman (2014). Alignments were generated separately in Geneious version 7.1.7 (Kearse et al. 2012) for each gene with MUSCLE (Edgar 2004) on the amino acid translation, back translated to DNA, and further adjusted manually. The *PHYP* gene was duplicated in Pinaceae into *PHYP1* and *PHYP2* (Schmidt and Schneider-Poetsch 2002; Mathews et al. 2010). Thirty-eight *PHYP* sequences were included: 23 Pinaceae *PHYP1*, nine representatives of *PHYP2*, and six outgroups. The *PHYP* alignment was 1969 bp, with an average of 1772 unambiguous sites per taxon (range 0%–61.8% missing). Twenty-nine taxa including six outgroups were represented by plastid sequence. The plastid DNA alignment was 59 193 bp, with an average of 50 104 unambiguous sites per taxon (range 0%–26.2% missing). Alignments are available from TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S19389).

Phylogenetic analyses

Parsimony analysis for morphology, nuclear *PHYP*, and plastid exons was performed on individual and combined matrices with and without fossils using TNT version 1.1 (Goloboff et al. 2008*b*). The number of variable and informative characters was determined with PAUP* version 4.0a147 (Swofford 2002). For DNA sequence alignments, inferred insertion–deletion events (gaps) were treated as missing data. Heuristic searches were performed with TNT using a combination of sectorial searches, tree-drifting, ratchet, and tree-fusing to quickly search for different islands and find the shortest trees; the search was terminated when the same tree length was found in 20 independent replicates ("xmu hit 20 drift 10"; Goloboff 1999; Nixon 1999). This strategy increased the likelihood of recovering the principal tree topologies. The resulting collections of most parsimonious trees (MPTs) were subjected to a round of tree-bisection–reconnection before calculating the strict consensus tree. Branch support was estimated using 1000 bootstrap replicates in TNT, each involving 10 replicates building Wagner trees with random addition sequence, followed by tree-bisection–reconnection branch swapping; bootstrap values ≥70% were considered strongly supported (Hillis and Bull 1993). Trees were converted to graphics with FigTree (Rambaut 2009).

To identify and prune taxa with an unstable position (= wildcard taxa) among equally optimal trees under equally weighted parsimony, we used the reduced consensus (positional congruence reduced; PCR) technique of Pol and Escapa (2009). The method was implemented in TNT ("pcrprune") after performing heuristic searches. We also evaluated the effect of deleting particular fossil taxa a priori, based on the hypothesis that those described from seed cones lacking scale apices or lacking seeds would have an unstable phylogenetic placement. Congruence among the three data sources was evaluated by inspecting the trees resulting from individual analysis for conflicting clades with bootstrap values ≥70% (Mason-Gamer and Kellogg 1996) and by applying the incongruence length difference (ILD) test (Farris et al. 1995); this was performed with 1000 replicates in PAUP* after excluding phylogenetically uninformative characters.

Implied character weighting (Goloboff 1993) was used in TNT to assign weights that were inversely proportional to their homoplasy, with the expectation that this would reduce error in phylogenetic estimation, and recover a more robust (i.e., with higher branch support) consensus tree. This method also tends to find fewer MPTs and therefore returns a more resolved consensus tree because of the high precision of the weighting calculations (Goloboff et al. 2008*a*). Heuristic searches were performed in TNT across a wide range of concavity values (k = 1–25) for separate and combined morphology, *PHYP*, and plastid partitions. The lowest concavity values correspond to the strongest downweighting of homoplasious characters. Bootstrap analyses were performed to calcu-

Table 1. Summary of parsimony analyses with characters weighted equally.

Description	Fossils	Terminals	Chars.	PI	Trees	L	CI	RI
Morphology	No	29	158	137	7	445	0.438	0.654
PHYP	No	38	1969	715	2	2491	0.640	0.715
Plastid exons	No	29	59193	10021	1	32753	0.729	0.743
Morphology, *PHYP*, plastid	No	29	61320	10693	1	35061	0.721	0.738
Morphology	Yes	75	158	158	4526	644	0.328	0.652
Morphology, *PHYP*, plastid	Yes	75	61320	10705	68	35257	0.718	0.737

late the average branch support obtained for each concavity value. In one case bootstrap and jackknife values were compared. The effect of different weighting parameters on the average bootstrap was graphed in the R environment (R Core Team 2014). We also used extended implied weighting to apply separate weights to the morphology, *PHYP*, and plastid subsets (Goloboff 2013). Each partition was assigned the concavity value that resulted in the highest average bootstrap (or jackknife) based on separate analyses of the extant taxa only. Fossil taxa that changed their position relative to living genera across different concavity values for the morphology partition were noted for further revision of character scoring.

For Bayesian inference, nucleotide substitution models for *PHYP* and the plastid exon alignments were chosen using jModelTest version 2.1.4 (Darriba et al. 2012). A maximum likelihood tree was estimated with PhyML (Guindon and Gascuel 2003) and log likelihood scores were calculated for 24 substitution models. The Mk model was applied to the morphological data (Lewis 2001). Bayesian analyses were performed in MrBayes version 3.2.2 (Ronquist et al. 2012*b*). Partition parameters were unlinked, and two runs of two to five million MCMC generations were run for each analysis. The first 25% of the trees resulting from the MCMC were excluded prior to burn-in; the remaining trees were summarized with a majority-rule tree, and the resulting taxon bipartition frequencies were reported as the posterior probability (PP) support for clades. Convergence was verified by assuring that average standard deviations of split frequencies between the two runs was <0.01, that effective sample sizes were >100, and by visual inspection of the log-likelihood-generation trace plots using Tracer version 1.6 (Rambaut et al. 2014).

Results

Relationships among extant species only

The morphology, plastid, and *PHYP* partition lengths were 158, 1969, and 59 193 characters, respectively, giving a total of 61 320 characters for the combined matrix (Table 1). The ensemble character consistency index (CI) was higher for *PHYP* and plastid DNA sequences than for morphology when each partition was considered separately (Table 1), as were CIs per individual character (ci) on the combined tree (mean and standard deviation of the consistency index excluding uninformative characters: *PHYP* = 0.648 ± 0.238, plastid = 0.698 ± 0.271, morphology = 0.505 ± 0.368; mean and SD of the retention index: *PHYP* = 0.588 ± 0.371, plastid = 0.664 ± 0.377, morphology = 0.319 ± 0.365). The consistency index for informative characters on the combined tree was slightly higher for seed cones and seeds (characters 75–154; ci = 0.520 ± 0.30) than for vegetative characters (characters 1–61; ci = 0.509 ± 0.26), whereas the retention index was slightly higher for vegetative characters (0.538 ± 0.35 versus 0.497 ± 0.382). The Akaike Information Criterion indicated that the best model for both *PHYP* and the plastid exons was the general time reversible model with a proportion of invariant sites and gamma distributed rate variation (GTR + I + G).

The morphology consensus tree was well resolved with parsimony and Bayesian analysis, but few branches received strong support (three branches ≥ 70% bootstrap/four branches ≥ 0.95 PP; Figs. 1A and 2A). The *PHYP* consensus trees were resolved at most nodes, with most branches receiving strong support (27 branches ≥ 70% bootstrap/28 branches ≥ 0.95 PP; Figs. 1B and 2B). The plastid consensus trees were completely resolved and most nodes received strong support (24 branches ≥ 70% bootstrap/26 branches ≥ 0.95 PP; Figs. 1C, 2C), but the placement of *Cathaya* differed between parsimony and Bayesian analyses (see below). The plastid and combined parsimony trees were identical, as were the plastid and combined Bayesian trees (22 branches ≥ 70% bootstrap/26 branches ≥ 0.95 PP; Figs. 1D and 2D).

Extant Pinaceae was recovered as monophyletic for morphology and all molecular data sets (bootstrap = 100%/PP = 1; Figs. 1 and 2, respectively). Pinoid genera (*Pinus*, *Picea*, *Cathaya*, *Larix*, and *Pseudotsuga*) were also monophyletic for all datasets (combined 100% bootstrap/PP = 1; Figs. 1 and 2). Abietoid genera (*Abies*, *Keteleeria*, *Cedrus*, *Pseudolarix*, *Tsuga*, and *Nothotsuga*) were recovered as monophyletic with morphology with low branch support (bootstrap < 70%/PP < 0.95) and in the Bayesian plastid and combined trees with high support (PP = 1; Figs. 2C and 2D). In contrast, *Cedrus* was sister to the remaining taxa of Pinaceae in the *PHYP*, plastid, and combined parsimony trees with low support (Figs. 1B–1D) and sister to the pinoid clade in the *PHYP* Bayesian tree with low support (Fig. 2B). The relationships among *Cathaya*, *Picea*, and *Pinus* also varied by analysis and dataset. Parsimony analyses recovered (*Picea*, (*Cathaya*, *Pinus*)) for the separate and combined datasets (Fig. 1), whereas all Bayesian

Fig. 1. Trees resulting from parsimony analysis of extant species (A) Morphology. Strict consensus of seven MPTs. (B) Strict consensus of two MPTs based on nuclear *PHYP*. (C) Single MPT based on plastid DNA sequence. (D) Single MPT based on morphology, nuclear *PHYP*, and plastid DNA sequence combined. Bootstrap values >50% (1000 reps) indicated at branches.

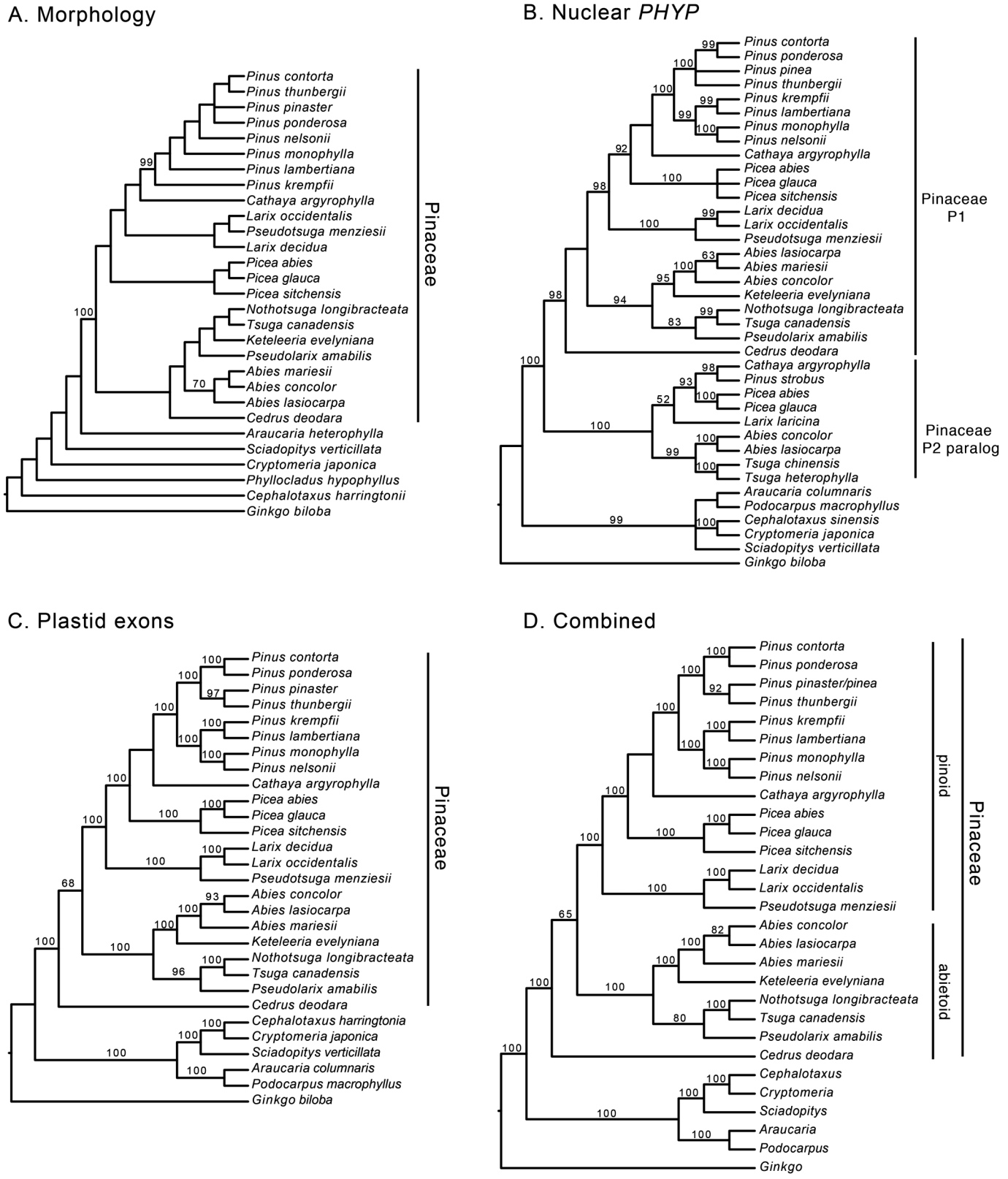


Fig. 2. Trees resulting from Bayesian analysis of extant species. (A) Morphology. (B) *PHYP*. (C) Plastid exons. (D) All sources combined. Posterior probability values >0.5 indicated at branches.

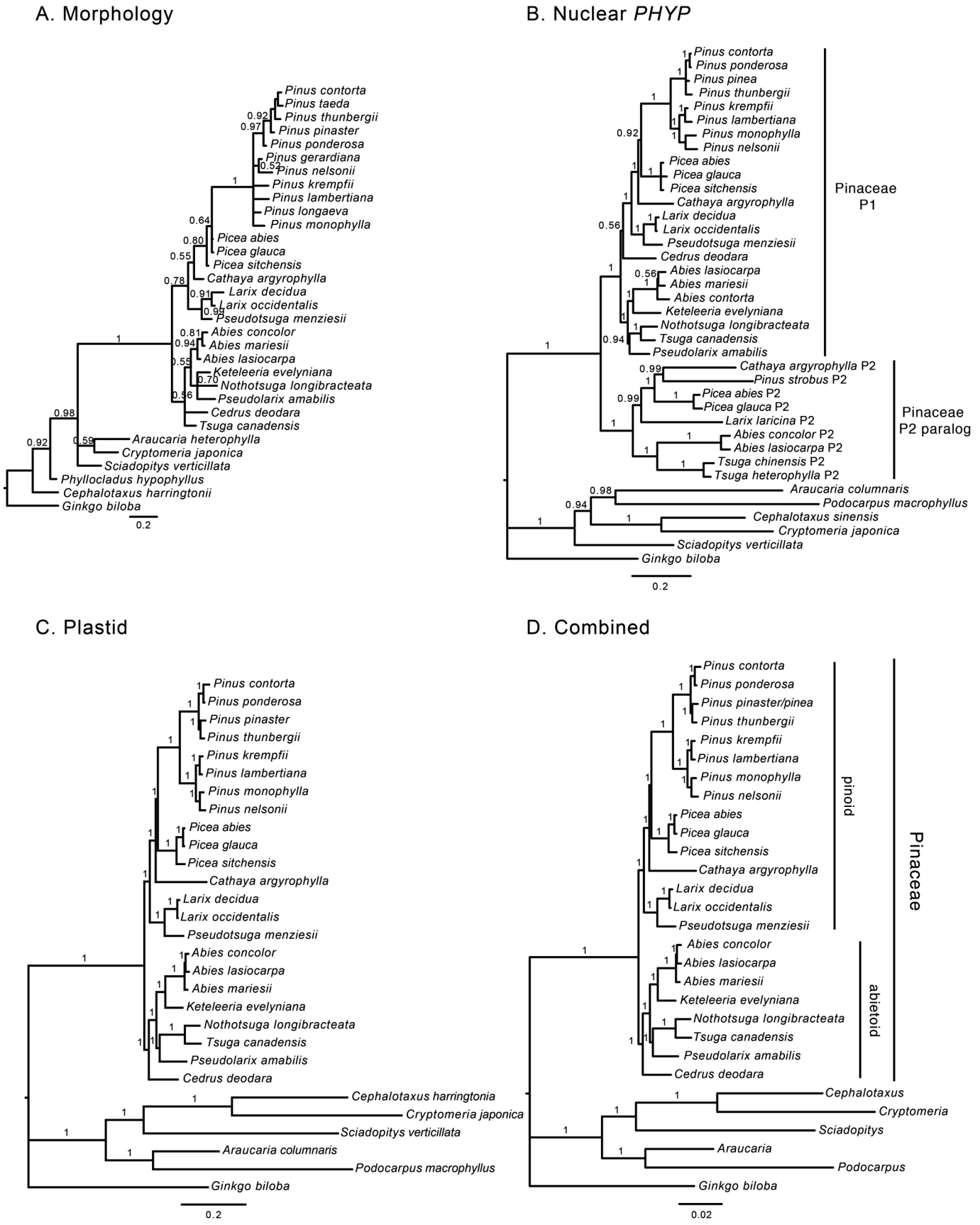
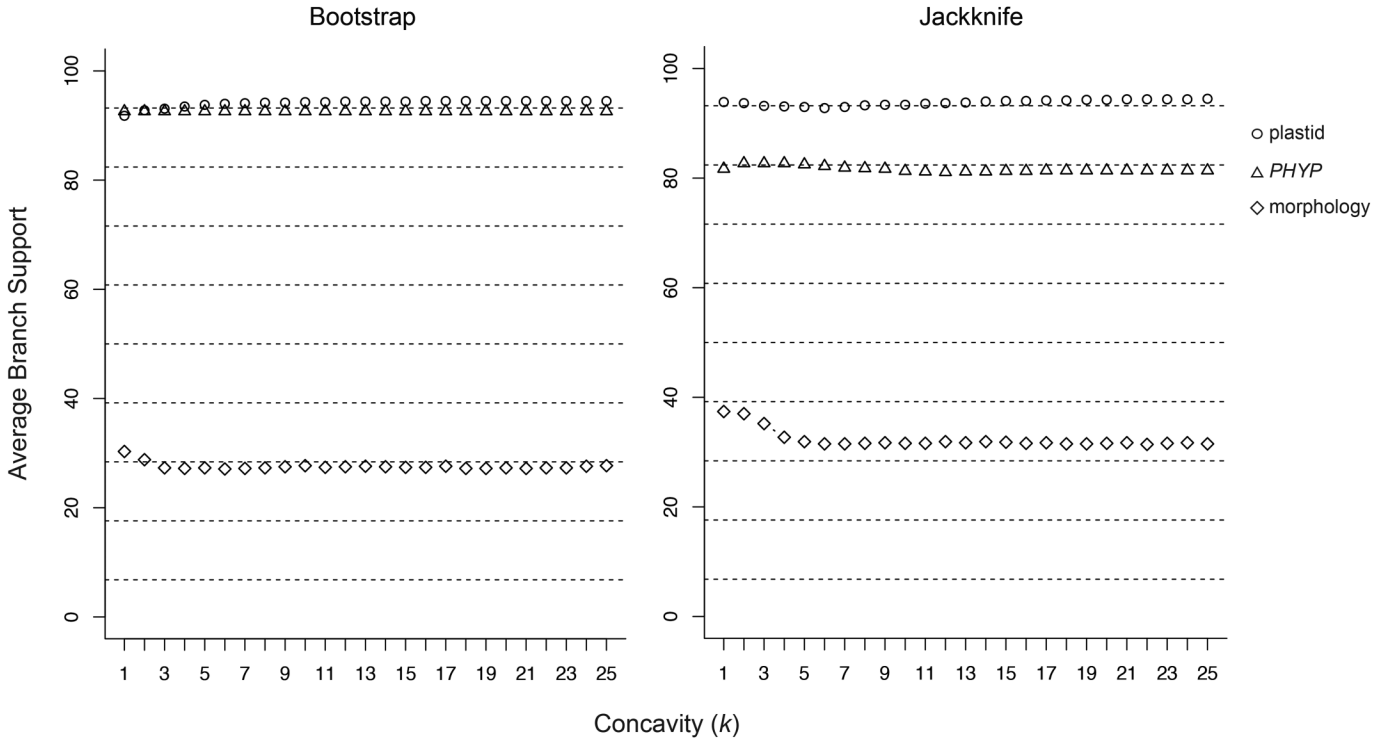


Fig. 3. The effect of varying the implied weight concavity value (k = 1–25) on average branch support for extant species. Left: bootstrap values. Right: jackknife values.



analyses recovered (*Cathaya*, (*Picea*, *Pinus*)) (Fig. 2). These relationships did not receive high branch support except in the combined Bayesian analysis. Other topological differences between *PHYP* and plastid relationships were that two of the eight species of *Pinus* were unresolved in the *PHYP* parsimony tree (Fig. 2B), as were the three species of *Picea* in both parsimony and Bayesian trees (Figs. 1B and 2B), and the position of *Sciadopitys* varied among the outgroup conifers in the *PHYP* parsimony and Bayesian trees (Figs. 1B, 1C, 2B, and 2C). Otherwise, molecular and combined trees (Figs. 1B–1D and 2B–2D) agreed for intergeneric relationships. Three genera of Pinaceae represented by multiple species (*Abies*, *Picea*, and *Pinus*) were each recovered as monophyletic in all analyses (Figs. 1 and 2). *Larix*, represented by two species, was paraphyletic with *Pseudotsuga* in the morphology parsimony tree (Fig. 1A) but was monophyletic in all other trees. In the morphology trees, only the *Pinus* clade received strong support. Support for monophyly of *Abies* and *Picea* was lower (Figs. 1A and 2A).

For the parsimony analyses, none of the topological differences between different data sources received strong branch support. The nuclear *PHYP* and plastid exon data matrices passed the ILD test (P = 0.900000), but comparisons between morphology and molecular partitions did not (*PHYP*: P = 0.000100; plastid exons: P = 0.000100). The Bayesian analyses recovered two relationships with high branch support that conflicted with poorly supported topologies in the parsimony trees: the abietoid genera were monophyletic for morphology, plastid and combined analyses, and *Picea*, rather than *Cathaya*, was sister to *Pinus* for all analyses (Fig. 2). Applying backbone constraint trees on extant taxa to enforce monophyly of Pinaceae and the abietoid and pinoid clades under parsimony resulted in a *PHYP* tree that was two steps longer and a plastid tree that was 8 steps longer than the unconstrained tree.

When using parsimony with implied weighting on the extant taxon data set and varying the concavity value (k), the morphology data attained the highest average bootstrap and jackknife support values with the strongest weights, corresponding to the lowest values of k (the maximum branch support was obtained for k = 1). Support values for the molecular partitions did not respond as markedly to different weighting strengths (Fig. 3). For the *PHYP* data, the average bootstrap value did not vary, but the maximum jackknife value was obtained at relatively low concavity values (k = 2–4). For the plastid data, there was a slightly negative relationship between support values and strength of weighting. Most relationships were insensitive to the strength of weighting, with the exception of the position of *Cedrus* and the relationships among *Cathaya*, *Picea*, and *Pinus*. Increased weighting strength tended to increase topological congruence with the Bayesian trees (Fig. 2). Abietoid genera were recovered as monophyletic for the plastid dataset when employing the strongest weighting ($k \leq 5$; the same topology as the Bayesian tree, Fig. 2B), but paraphyletic (*Cedrus* sister to Pinaceae) with weaker weighting. The relationship ((*Cathaya*, *Picea*), *Pinus*), which was not recov-

ered in either equally weighted or Bayesian analyses, was recovered with strong weighting ($k \leq 5$), whereas the relationship (*Picea*, (*Cathaya*, *Pinus*)) was recovered with weaker weighting. For the combined data, abietoid genera again were recovered as monophyletic when employing the strongest weighting ($k \leq 3$), but paraphyletic (*Cedrus* sister to Pinaceae) with weaker weighting. The relationship (*Cathaya*, (*Picea*, *Pinus*)) was recovered with strong weighting ($k \leq 3$), but the relationship (*Picea*, (*Cathaya*, *Pinus*)) with weaker weighting. For *PHYP*, *Cedrus* was sister to pinoid genera for $k = 1$ (same topology as Bayesian tree; Fig. 2B), and sister to Pinaceae for all higher concavity values; the (*Cathaya*, (*Picea*, *Pinus*)) topology was recovered except under the weakest weighting ($k \geq 22$ and equal weights), which recovered (*Picea*, (*Cathaya*, *Pinus*)).

Relationships among extant and fossil species

When including fossil species, equally weighted parsimony analyses of morphology recovered 4526 MPTs for morphology (the maximum limit of trees was increased from 1000 to 5000 for this search; L = 644, CI = 0.328, RI = 0.652; Supplementary data, Fig. S3). A total of 68 MPTs was recovered for the combined dataset (L = 35 257, CI = 0.718, RI = 0.737; Table 1; Fig. 4A). Combining the molecular data available for the living taxa resulted in a more resolved strict consensus compared to morphology alone (42 versus 18 nodes resolved), with more branches receiving moderate to high bootstrap support (16 branches with bootstrap support ≥ 70% in the combined tree compared with four branches in the morphology tree; Fig. 4A). Pinaceae was not resolved as monophyletic in the morphology or the combined tree. In the morphology tree, Pinaceae formed a polytomy with *Araucaria*, *Cryptomeria*, and *Sciadopitys*, whereas in the combined tree, *Eathiestrobus mackenziei* formed a polytomy with the conifer outgroups and *Pararaucaria patagonica* (Cheirolepidiaceae) occurred within Pinaceae as sister to a *Pseudoaraucaria* clade. The genera *Abies*, *Picea*, *Obirastrobus*, and *Pseudoaraucaria* were each recovered as monophyletic in both the morphology and combined trees, whereas *Pinus* formed a clade with six species of *Pityostrobus* in the morphology tree but mainly collapsed in the combined tree. *Abies*, *Keteleeria*, *Nothotsuga*, *Tsuga*, and *Pseudolarix* formed a clade in both the morphology and the combined tree, as did *Pseudotsuga* and *Larix*. Most other deep intergeneric relationships were unresolved; this was caused by several fossil taxa having dramatically unstable positions across optimal trees, leading to polytomies in the strict consensus.

Applying the iterative positional congruence method to the best trees resulting from the equally weighted combined analysis identified 11 unstable taxa (9 unstable terminal taxa and one node with two taxa; Fig. 4A). Pruning these taxa completely resolved relationships in the reduced consensus tree, which recovered abietoid genera as paraphyletic (Fig. 4B).

The combined dataset with all 75 taxa was analysed using implied weights (1) by applying a single concavity constant for both morphology and the combined matrix that varied from $k = 1$–25, and (2) using extended implied weighting to apply a fixed concavity constant for *PHYP* ($k = 3$) and plastid DNA ($k = 13$) but variable constant for morphology ($k = 1$–20). Weighting resulted in fewer MPTs and a correspondingly better-resolved consensus across all concavity values. When applying a single concavity constant for morphology alone, the highest average bootstrap was obtained for $k = 8$ (16.6% compared with 14.4%–16.5%). For the combined matrix, $k = 15$ resulted in the highest average bootstrap support (33.0% compared with 31.8%–32.4%; not shown). Relationships among extant taxa were identical to those found when no fossils were included for plastid, *PHYP*, and combined matrices, differing only in whether abietoid genera were monophyletic (low values of k) or paraphyletic with *Cedrus* sister to all of Pinaceae, and in the relationships among *Cathaya*, *Picea*, and *Pinus* (intermediate and high values of k). The fossil genus *Obirastrobus* was consistently recovered as monophyletic, as was *Pseudoaraucaria* except at the lowest concavity value ($k = 1$). The position of *Pityostrobus* species, *Picea burtonii*, *Eathiestrobus mackenziei*, and *Pararaucaria patagonica* varied with respect to the extant taxa.

Five fossil species have missing data for the presence or absence of a cone scale umbo, or for umbo position: *Pityostrobus argonnensis*, *P. macrocephalus*, *P. palmeri*, *P. shastaensis*, and *Pseudoaraucaria benstedii*. Four other fossils lack data for seeds: *Pityostrobus jacksonii*, *P. lynnii*, *P. milleri*, and *P. ramentosa*. Deleting these nine species did not result in a more resolved consensus tree using parsimony with equal weights (Supplementary data, Fig. S5). Applying the iterative positional congruence method to the trees identified 25 unstable fossil taxa for pruning (Supplementary data, Fig. S5). Use of extended implied weights ($k = 3$ *PHYP* and $k = 13$ plastid) resulted in well-resolved consensus trees across a wide range of concavity values for morphology. The maximum average bootstrap was attained for $k = 22$ for morphology, with 22 branches having support ≥ 70% (Fig. 5A), compared with 16 branches when all fossil taxa were included (Fig. 4A). In other words, deleting nine taxa resulted in an increase in branches with bootstrap support ≥ 70%. Pinaceae was recovered as monophyletic; its earliest branching lineage was *Eathiestrobus mackenziei* across all concavity values except $k = 2$. Abietoid genera were paraphyletic to pinoid genera. The fossil genus *Obirastrobus* was recovered as monophyletic at all concavity values, and *Pseudoaraucaria* was recovered as monophyletic across all but the lowest concavity value ($k = 1$). The fossil *Picea burtonii* was recovered in the pinoid clade at lower concavity values ($k = 1$–3), including in a trichotomy with extant *Picea* and a *Cathaya*, *Pinus*, and *Pityostrobus* clade ($k = 2$), whereas it occurred in a clade that included *Pseudoaraucaria*, *Obirastrobus*, and two species of *Pityostrobus*

Fig. 4. Strict consensus trees resulting from parsimony analyses of extant and fossil species based on (A) combined morphological and molecular characters. Taxa indicated with an asterisk (*) were identified for pruning by the iterative reduced consensus method. (B) Reduced consensus.

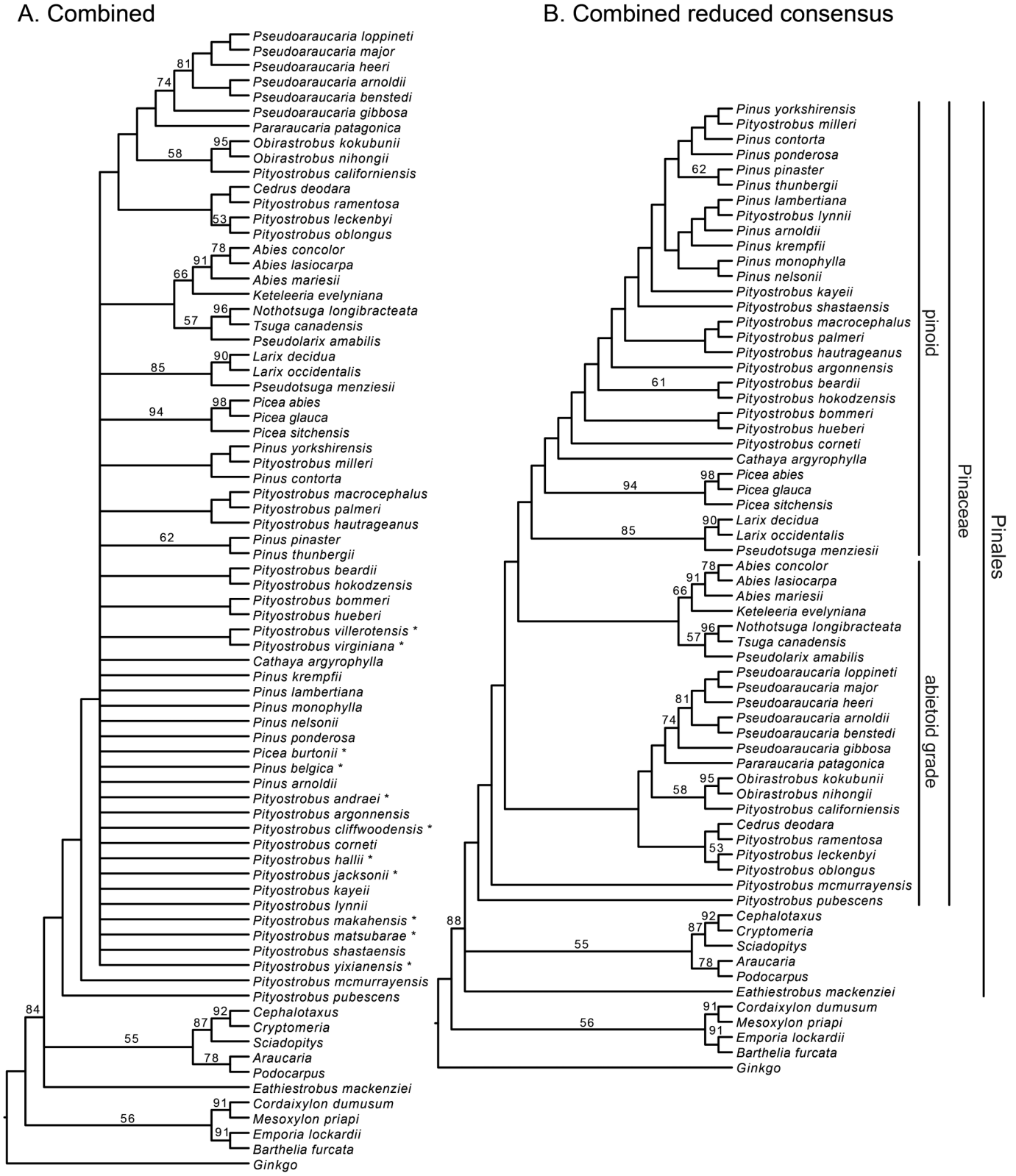


Fig. 5. Consensus trees resulting from extended implied weighting of all taxa except those lacking seed cone scale apices or seeds. (A) Strict consensus of six MPTs (L = 2304.64036, CI = 0.718, RI = 0.736; *k* = 22 morph, = 3 *PHYP*, = 13 plastid). Bootstrap values >50% (1000 reps) indicated above branches. Fossil taxa with a single asterisk (*) were unstable within either the abietoid grade or the pinoid clade as the weighting strength was varied (*k* = 1–25). Fossils with a double asterisk (**) were unstable between the abietoid grade and pinoid clade as the weighting strength was varied. (B) Reduced consensus tree of same analysis (A) with 12 unstable taxa pruned. Key to symbols used for characters in 5B: rv, resin vesicles (open circles, absent; closed circles, present; character 144); su, ovuliferous scale umbo (open circles, absent; closed circles, present; character 98); st, bract and scale trace origin (open circles, separate; closed circles, united; character 107); vb, ovuliferous scale vascular bundle shape distal to seed (open circles, straight; closed circles, adaxially convex; character 121); rc, scale resin canals at base of cone (open circles, absent; closed circles, present; first column, abaxial to scale trace; second column, adaxial; characters 124 and 125, respectively).

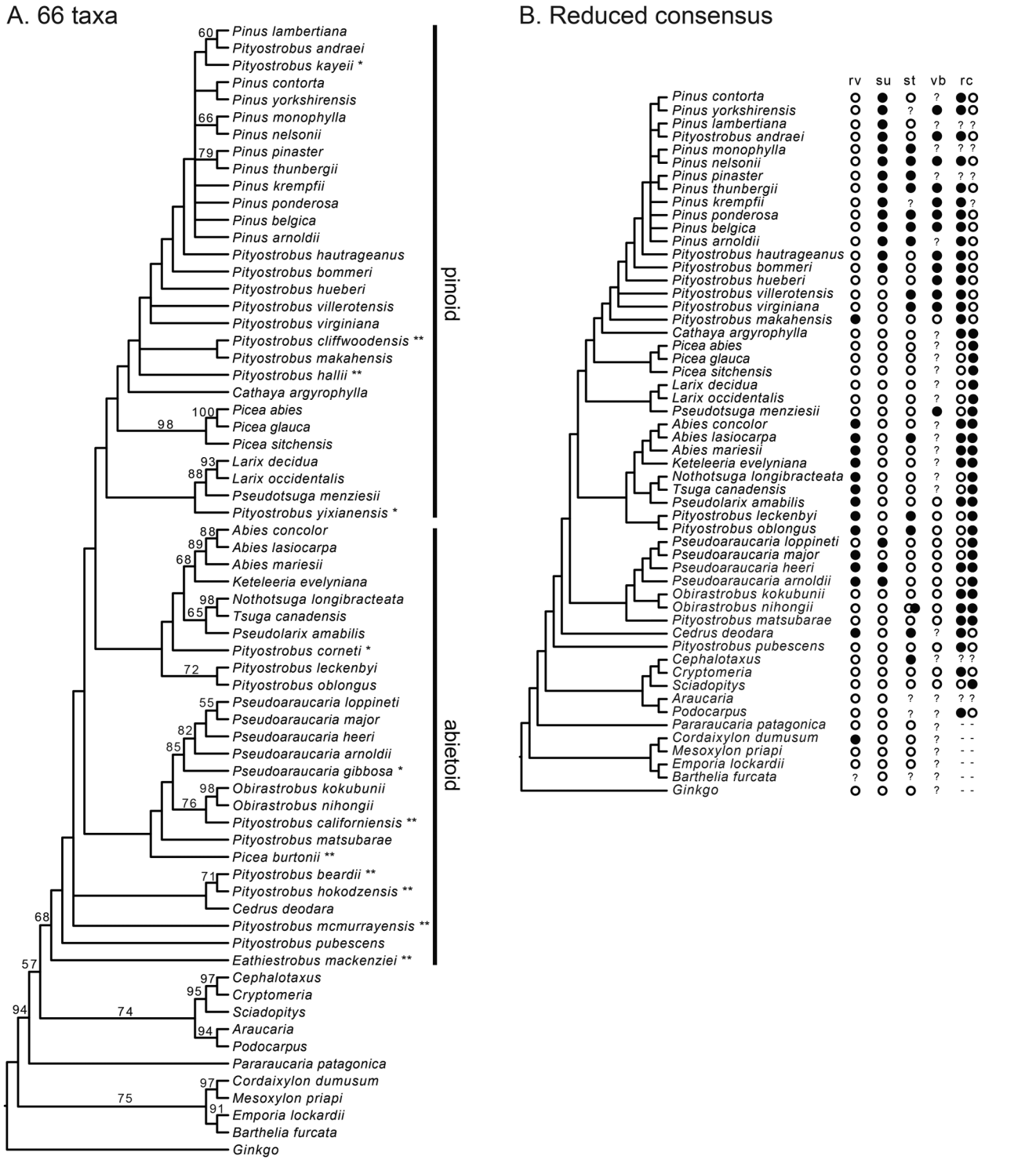


Fig. 6. Majority rule consensus tree from Bayesian analysis of combined data. Fossils missing cone scale apices or seeds, or identified as unstable with parsimony, were excluded from the analysis. Posterior probabilities are indicated above branches.

at intermediate and higher concavity values ($k \geq 5$). The placement of *Pityostrobus hokodzensis*, *P. beardii*, *P. californiensis*, and *P. mcmurrayensis* changed from the abietoid grade to the pinoid clade at the lowest concavity value, and *P. makahensis* changed from the pinoid clade to the abietoid grade.

A Bayesian analysis of the combined dataset and including the 48 taxa with a stable position when using implied weighting recovered Pinaceae as monophyletic in the majority rule consensus tree, as well as all extant genera represented by multiple species, *Pseudoaraucaria*, and *Obirastrobus* (Fig. 6). Most other relationships were poorly resolved. Eight nodes received strong support (PP ≥ 0.95).

Discussion

Relationships among extant taxa

The relationships among genera inferred by separate analyses of molecular and morphological trees agree in many aspects. Monophyly of extant Pinaceae is strongly supported by morphological and molecular evidence, corroborating previous phylogenetic studies (Wang et al. 2000; Gernandt et al. 2008; Lin et al. 2010; Ryberg et al. 2012; Lu et al. 2014). The division of the family into two groups first proposed by Van Tieghem (1891), based on the presence of one (abietoid) or more than one (pinoid) resin canal in the vascular cylinder of the taproot, is also supported by the presence of resin cavities in the seed integument of the abietoid taxa. Monophyly of the pinoid group is well supported by both morphological and molecular data, but abietoid genera are often recovered as paraphyletic (see below). Other intergeneric relationships based on combined analysis of nuclear and plastid DNA and morphology result in high resolution and branch support within the family, and are consistent with both traditional, morphology-based classifications and molecular systematic studies. Trees disagree in the position of

Cedrus (which affects the placement of the root of the family), and in the interrelationships among *Cathaya*, *Picea*, and *Pinus*, and (Figs. 1 and 2). Regarding the circumscription of genera represented by more than one species in the analyses, *Pinus* and *Abies* are recovered as monophyletic with strong to moderate branch support in all trees, and *Picea* and *Pseudotsuga* are recovered as monophyletic in molecular trees.

The position of the root to Pinaceae is arguably the most important disagreement among analyses and datasets. Previous analyses of plastid, mitochondrial, or nuclear DNA have either resolved the abietoid genera *Cedrus*, *Abies*, *Keteleeria*, *Tsuga*, *Nothotsuga*, and *Pseudolarix* as monophyletic or paraphyletic, with incongruence among analyses often involving the position of *Cedrus* (Wang et al. 2000; Gernandt et al. 2008; Lu et al. 2014). The resolution of this branch is sensitive to different data sources and types of analysis. Whereas neither alternate rooting is well supported by any individual data source, analyses that correct for homoplasy (implied weighting or Bayesian analyses with models that permit different rates of character transformation) weakly tend to recover abietoid genera as monophyletic. Alternatively, if abietoid taxa are paraphyletic, then some characters used to define abietoid genera could be plesiomorphic. Analyses of living taxa gave inconsistent results between parsimony and Bayesian trees, but Bayesian trees also were inconsistent among data sources (Figs. 1 and 2).

Instances of topological conflict between the morphological and molecular trees are evident, although the conflict is weakly supported. Many of the incongruences relate to the interrelationships within *Pinus*. Character conflict between the nuclear and plastid DNA partitions of Pinaceae were insignificant based on the ILD test, but comparisons between morphological and molecular datasets were significant, as previously reported (Gernandt et al. 2008). The morphological dataset has fewer characters, and on average these have lower consistency and retention indices than the molecular characters. In contrast, molecular results capture relationships first suggested by morphology, such as generic concepts and the recovery of pinoid genera as monophyletic as suggested by seed morphology and root anatomy.

The 158 morphological, anatomical, and biochemical characters included in this study represent the largest structural data set analyzed to date for Pinaceae. More than a third of the characters were first used in a cladistic analysis of conifer genera (Hart 1987), the earliest phylogenetic analysis to address relationships within this family. Additional cone characters for Pinaceae were proposed later (Alvin 1988; Smith and Stockey 2001; Gernandt et al. 2008; Ryberg et al. 2012). The number of taxa analysed has also increased. For example, Hart (1987) included ten (generic) terminals for Pinaceae, and 38 of the 123 characters included in his analysis were variable within Pinaceae. Ryberg et al. (2012) included 48 Pinaceae terminals (including 11 extant taxa) and 56 characters, all from the seed cone. In the present study, roughly two-thirds of the morphological characters analysed are vegetative, embryological, or from pollen cones. With the exception of *Pinus arnoldii*, these characters are unknown for the Pinaceae fossil taxa that have been the focus of phylogenetic analyses (e.g., Alvin 1988; Smith and Stockey 2001, 2002; Gernandt et al. 2008; Klymiuk et al. 2011; Ryberg et al. 2012). A substantial number of anatomical and embryological characters are scored as missing data for most living taxa. More comparative studies are needed.

The lower character consistency for morphology can be attributed to a combination of higher real levels of homoplasy, and our imperfect understanding of character homologies and transformations in the group. The latter highlights the importance of conducting more revisionary morphological and ontogenetic studies in conifers. Improved character concepts are needed and organismal features could be measured more precisely (e.g., with quantitative characters or geometric morphometry). This should provide more accurate character state scorings and reduce missing data. Pinaceae has been the focus of numerous ontogenetic studies, but taxonomic coverage is incomplete. Many of the morphological character concepts do not consider ontogeny (for example, shoot dimorphism, branch orientation, xylem pitting, and the formation and distribution of sclerenchyma).

Advances in inferring relationships among living and fossil Pinaceae

Robust hypotheses for the phylogenetic placement of fossil taxa are of great value for understanding the timing of divergence of higher groups and for reconstructing their ancestral character states. Adding fossils to the extant Pinaceae dataset drastically decreased both resolution and branch support (Fig. 4A), and led to a further drop in the ensemble consistency index for morphological characters. Adding vegetative characters known only for living taxa and DNA sequences increased average branch support (not shown), and identifying and pruning unstable fossil taxa from the consensus tree increased resolution (Fig. 4B). Implied weighting not only increased resolution of consensus trees but also increased branch support (Fig. 5). These strategies result in similar tree topologies for living Pinaceae, and allowed us to identify which fossils have a stable position.

Six of the seven genera represented by more than one species (all except *Pityostrobus*) are recovered as monophyletic in combined analyses. *Pinus*, *Abies*, and *Picea* have the greatest number of extant species in the family. When analysed without fossils, each of these genera is recovered as monophyletic with high branch support, giving the impression that their taxonomic limits are well understood. However, when analysed with fossils, *Picea burtonii*, described from a fossil cone, does not consistently group with extant *Picea*, rather its placement

within the family is unstable across different analyses, even occurring with abietoid genera with implied weighting (Fig. 5).

The fossil genus *Pityostrobus* is recovered as polyphyletic, consistent with its treatment as an artificial group (Miller 1976). It includes some close relatives or members of *Pinus*, and other species that group with abietoid genera. Instability in the inferred relationships between *Pityostrobus* taxa and *Pinus* causes an overall drop in branch support and a loss of resolution, for example for the two *Pinus* subgenera (*Pinus* and *Strobus*). However, of the seven species of *Pityostrobus* that form a clade with *Pinus* across all analyses reported here (Fig. 5), only two (*P. andraei* and *P. hautrageanus*) are interpreted as possessing the principal external cone morphological synapomorphies of *Pinus*: cone scales thickened apically and an umbo. Four (*P. hueberi*, *P. makahensis*, *P. villerotensis*, and *P. virginiana*) lack an umbo, and *P. bommeri* has a terminal umbo, but is not considered as having apically thickened scales. Terminal umbos only occur in *Pinus* subsection *Strobus*. The cone scale umbo is dorsal in the other 10 living subsections of *Pinus*. In subsection *Strobus* it is often difficult to perceive the thickening of the apical scales into an apophysis. Other characters indicated by Miller (1976) as diagnostic for *Pinus*, namely bract and scale traces united at their origin and scale traces that become curved distally in the cone scale, are variable in extant species and should not be used to exclude "pine-like" *Pityostrobus* species from the genus (Fig. 5; Gernandt et al. 2011; Ryberg et al. 2012).

Analyses of morphology alone and in combination with DNA support the delimitation of the fossil genera *Pseudoaraucaria* and *Obirastrobus*, which were erected as natural groups (Alvin 1988; Ohsawa et al. 1992), and were recovered as monophyletic in previous cladistic analyses (Smith and Stockey 2002; Gernandt et al. 2011; Ryberg et al. 2012). Both genera show some instability in the analyses; the position of *Pseudoaraucaria* varies among the abietoid genera, and one species, *Pseudoaraucaria gibbosa*, did not group with the remaining species under some weights (Supplementary data, Fig. S4). The two species of *Obirastrobus* are always recovered as monophyletic. They occur among abietoid genera in most trees, often grouping tenuously with *Pseudoaraucaria*.

Parsimony with implied weights recovered *Cedrus* together with several fossil taxa (most commonly *Pseudoaraucaria*, *Obirastrobus*, and *Pityostrobus californiensis*) forming a paraphyletic grade leading to a clade of the remaining abietoid taxa, but without strong branch support. This is consistent with the fossil taxa belonging to the Pinaceae crown group, but would be clearer if the phylogenetic position of *Cedrus* were more robust. The abietoid genera were recovered as monophyletic when applying stronger weights, particularly for the molecular data.

The genus *Eathiestrobus* was erected for a Late Jurassic seed cone, the earliest that can be assigned with confidence to Pinaceae (Rothwell et al. 2012). Under some weights it is recovered as sister to Pinaceae, but under others it is sister only to the pinoid clade. Like all members of the pinoid clade, *Eathiestrobus* has persistent cone scales and lacks resin vesicles in the seed integument. Abietoid genera have resin vesicles in the seed integument, and in some genera, disarticulating seed scales. It is uncertain which of these states are plesiomorphic for Pinaceae. If abietoid genera really do form a monophyletic group, then disarticulating seed scales and resin vesicles in the seed integument could be synapomorphies, and the lack of these characters in pinoid genera would reflect the ancestral state for Pinaceae. In this case pinoid genera and *Eathiestrobus* could be sharing ancestral character states, which would not require them to be monophyletic. Alternatively, if disarticulating seed scales and resin vesicles in the seed integument are ancestral in the family, then either *Eathiestrobus* should be sister to pinoid genera, sharing with them the derived character states, or then these putatively abietoid character states have been lost independently in *Eathiestrobus* and the pinoid genera. Identifying relatives of Pinaceae in the fossil record would help us to understand the early character evolution of the family, how the family evolved from the most recent common ancestor of conifers, and provide clues to the divergence of other seed plants (Mathews 2009; Rothwell et al. 2012).

Bayesian analyses recover relationships among living and fossil Pinaceae similar to those based on parsimony, with a dramatic loss in resolution in the majority-rule tree when fossils are added (Fig. 6). Both kinds of analyses agree in the monophyly of the family, the abietoid affinity of *Pseudoaraucaria*, and the polyphyly of *Pityostrobus*, with some species closely related to *Pinus*. The Bayesian consensus tree serves as an important contrast to the increased resolution that can be obtained with implied weights strict consensus trees (Fig. 5), and as a reminder that the statistical support is low for many relationships of great interest, regardless of analytical method. Bayesian total evidence approaches that incorporate fossil age information (Ronquist et al. 2012*a*) offer analytical options for these data that have yet to be explored.

Selecting representative fossil taxa for inclusion in phylogenetic analyses

Simulation studies have suggested that analyses of matrices with too many incomplete fossil taxa tend to result in many equally optimal trees and a reduction in accuracy of phylogenetic estimation (Wiens 2006). Empirical results from phylogenetic analyses of Pinaceae morphological matrices support this conclusion. For the dataset of Smith and Stockey (2002), 35 of the 48 taxa (78%) are fossils. Using a reduced consensus method, Pol and Escapa (2009) identified 14 taxa in this matrix as unstable. However, among the unstable taxa were species-rich extant generic terminals such as *Pinus*, which had many of its characters scored as polymorphic. Ideally, the

choice of which fossils to include, or which to exclude a priori or prune a posteriori, should be explicit and evidence-based. Here, we evaluated different strategies for selecting (or excluding) fossils, namely performing an analysis with all available taxa and pruning those that are unstable in equally optimal trees, analysing matrices with different weighting strengths and identifying taxa that are unstable across different weights, and deleting taxa that are missing consistent phylogenetically informative characters (cone scale apices and seeds). Of the three strategies, the only one that consistently improved overall branch support was implied weighting. However, using the three strategies in combination achieved the best resolved consensus tree with relatively high branch support (Fig. 5B). This tree supports the monophyly of Pinaceae, allows full resolution of living taxa, and locates some fossil taxa in a paraphyletic abietoid grade, and other species of *Pitystrobus* in a clade with *Pinus*. Four of the seven species recovered in the *Pinus* clade were among species identified as "pine like" in previous phylogenetic studies (Gernandt et al. 2011; Ryberg et al. 2012).

Upon varying the weight applied to characters in the combined dataset that included fossils, we observed that the phylogenetic relationships among extant taxa were stable except for the position of the root and the interrelationships among *Pinus*, *Picea*, and *Cedrus*. It was also possible to identify 12 fossil species whose position is unstable with respect to the living genera (Fig. 5). Eight of these unstable fossils are classified as *Pityostrobus*. These species can be considered as possessing combinations of characters that do not occur in living taxa (Miller 1976), although alternatively, some characters may have been misinterpreted in the limited material available for study. The other taxa identified as unstable were *Eathiestrobus mackenziei* (discussed above), *Picea burtonii*, and to a much lesser degree, *Pseudoaraucaria gibbosa*. The unstable position of *Picea burtonii* might indicate a need to identify diagnostic characters for the genus. Instability of *Pseudoaraucaria* might similarly indicate a need to better identify diagnostic characters, together with a relatively high amount of morphological variability in the genus.

Conclusions

Pinaceae left a record of complex, anatomically preserved fossil organs extending to the Lower Cretaceous and presumably to the Jurassic. Including more than 40 fossils in phylogenetic analyses using equally weighted parsimony or Bayesian analyses of morphological or combined morphological and molecular characters results in poorly resolved consensus trees with low branch support, but overall the results of these analyses have agreed in placing many of these fossils in the Pinaceae crown group. This suggested that the living genera diverged during or prior to the Lower Cretaceous.

Use of implied weighting, alone or in combination with selecting a stable subset of fossils, increases resolution and branch support, although lower resolution is recovered with Bayesian analysis. The implied weighting results agree with previous analyses of fossil and living taxa in resolving the oldest fossils (e.g., the *Pseudoaraucaria* and *Pinus* fossils from the Lower Cretaceous) in phylogenetically distant parts of the tree (Alvin 1988; Gernandt et al. 2008, 2011; Ryberg et al. 2012), consistent with a sudden appearance of both pinoid and abietoid lineages in the Lower Cretaceous. The phylogenetic position of *Eathiestrobus mackenziei*, the oldest known pinaceous taxon, (153–155.6 Ma), is unstable; it may belong to either the crown or stem group (the sister group) of extant Pinaceae. Estimates from molecular clocks have placed the crown group age of Pinaceae in the Jurassic (Wang et al. 2000; Gernandt et al. 2008; Lin et al. 2010; Leslie et al. 2012; Lu et al. 2014). More robust phylogenetic hypotheses will be achieved by identifying more morphological characters for inclusion and by critically evaluating their formulation and the accuracy of scoring their alternative states. Further morphological and anatomical revisions of representative conifers should improve both tree inference and our understanding of character evolution.

Acknowledgements

The authors thank Steffi Ickert-Bond for providing seed cone sections of *Pinus krempfii*, Berenit Mendoza, Instituto de Biología for assistance with SEM images, Cecelic Reséndiz Arias, Jeffrey Boore, and Jennifer Kuehl for laboratory assistance, Damon Little and Ignacio Escapa for helpful discussions of character coding and analysis, and Rich Cronn for advice on a previous version of the manuscript. This project was funded by UNAM DGAPA-PAPIIT grants (IN228209) to David Gernandt, by US National Science Foundation grants (ATOL-0629508) to Aaron Liston and Rich Cronn, (AToL-0629713) to Chris Campbell, (AToL-0120709) to Linda Raubeson, and (EF-0629819) to Gar Rothwell and Ruth Stockey, and the Maine (U.S.A.) Agricultural and Forest Experiment Station.

References

Alvin, K.L. 1960. Further conifers of the Pinaceae from the Wealden Formation of Belgium, etc. Pl. X. Bruxelles. p. 39.

Alvin, K.L. 1988. On a new specimen of *Pseudoaraucaria major* Fliche (Pinaceae) from the Cretaceous of the Isle of Wight. Bot. J. Linn. Soc. **97**(2): 159–170. doi:10.1111/j.1095-8339.1988.tb02458.x.

Baas, P., Schmid, R., and van Heuven, B.J. 1986. Wood anatomy of *Pinus longaeva* (bristlecone pine) and the sustained length-on-age increase of its tracheids. IAWA J. **7**(3): 221–228. doi:10.1163/22941932-90000988.

Bailey, I.W. 1909. The structure of the wood in the Pineae. Bot. Gaz. **48**(1): 47–55. doi:10.1086/329935.

Behnke, H.-D. 1974. Sieve-element plastids of *Gymnospermae*: their ultrastructure in relation to systematics. Plant Syst. Evol. **123**(1): 1–12. doi:10.1007/BF00983281.

Blokhina, N.I., and Afonin, M.A. 2007. Fossil wood *Cedrus penzhinaensis* sp. nov. (Pinaceae) from the Lower Cretaceous of north-western Kamchatka (Russia). Acta Paleobot. **47**(2): 379–389.

Chamberlain, C.J. 1935. Gymnosperms structure and evolution. The University of Chicago Press, Chicago, Illinois. p. 504. doi:10.5962/bhl.title.10239.

Cronn, R., Liston, A., Parks, M., Gernandt, D.S., Shen, R., and Mockler, T. 2008. Multiplex sequencing of plant chloroplast genomes using Solexa sequencing-by-synthesis technology. Nucleic Acids Res. **36**(19): e122. doi:10.1093/nar/gkn502. PMID: 18753151.

Darriba, D., Taboada, G.L., Doallo, R., and Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods, **9**: 772. doi:10.1038/nmeth.2109.

Donoghue, M.J., Doyle, J.A., Gauthier, J., Kluge, A.G., and Rowe, T. 1989. The importance of fossils in phylogeny reconstruction. Annu. Rev. Ecol. Syst. **20**: 431–460. doi:10.1146/annurev.es.20.110189.002243.

Doyle, J.J., and Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. **19**(1): 11–15.

Eckenwalder, J.E. 2009. Conifers of the world: the complete reference. Timber Press, Portland, Ore. p. 720.

Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. **32**(5): 1792–1797. doi:10.1093/nar/gkh340. PMID:15034147.

Escapa, I.H., Rothwell, G.W., Stockey, R.A., and Cúneo, N.R. 2012. Seed cone anatomy of Cheirolepidiaceae (Coniferales): reinterpreting *Pararaucaria patagonica* Wieland. Am. J. Bot. **99**(6): 1058–1068. doi:10.3732/ajb.1100544. PMID:22665438.

Escapa, I.H., Cúneo, N.R., Rothwell, G., and Stockey, R.A. 2013. *Pararaucaria delfueyoi* sp. nov. from the Late Jurassic Cañadón Calcáreo Formation, Chubut, Argentina: Insights into the evolution of the Cheirolepidiaceae. Int. J. Plant Sci. **174**(3): 458–470. doi:10.1086/668612.

Esteban, L.G., and De Palacios, P. 2009. Comparative wood anatomy in Abietoideae (Pinaceae). Bot. J. Linn Soc. **160**(2): 184–196. doi:10.1111/j.1095-8339.2009.00971.x.

Farjon, A. 1990. Pinaceae: drawings and descriptions of the genera *Abies*, *Cedrus*, *Pseudolarix*, *Keteleeria*, *Nothotsuga*, *Tsuga*, *Cathaya*, *Pseudotsuga*, *Larix* and *Picea*. Koeltz Scientific Books, Köenigstein, Federal Republic of Germany. p. 330.

Farjon, A. 2010. A handbook of the world's conifers. Brill Academic Publishers, Leiden, Netherlands.

Farris, J.S., Källersjö, M., Kluge, A.G., and Bult, C. 1995. Testing significance of incongruence. Cladistics, **10**(3): 315–319. doi:10.1111/j.1096-0031.1994.tb00181.x.

Forey, P.L., and Kitching, I.J. 2000. Experiments in coding multistate characters. *In* Homology and systematics: coding characters for phylogenetic analysis. Vol. 58. Systematics Association, London, UK. pp. 54–80.

Frankis, M. 1988. Generic inter-relationships in Pinaceae. Notes Roy. Bot. Gard. Edinburgh, **45**: 527–548.

García-Gil, M.R., Mikkonen, M., and Savolainen, O. 2003. Nucleotide diversity at two phytochrome loci along a latitudinal cline in *Pinus sylvestris*. Mol. Ecol. **12**(5): 1195–1206. doi:10.1046/j.1365-294X.2003.01826.x. PMID:12694283.

Gernandt, D.S., Magallón, S., Geada López, G., Zerón Flores, O., Willyard, A., and Liston, A. 2008. Use of simultaneous analyses to guide fossil-based calibrations of Pinaceae phylogeny. Int. J. Plant Sci. **169**(8): 1086–1099. doi:10.1086/590472.

Gernandt, D.S., León-Gómez, C., Hernández-León, S., and Olson, M.E. 2011. *Pinus nelsonii* and a cladistic analysis of Pinaceae ovulate cone characters. Syst. Bot. **36**(3): 583–594. doi:10.1600/036364411X583565.

Gerry, E. 1910. The distribution of the 'bars of Sanio' in the Coniferales. Ann. Bot. **24**(1): 119–123.

Goloboff, P.A. 1993. Estimating character weights during tree search. Cladistics, **9**(1): 83–91. doi:10.1111/j.1096-0031.1993.tb00209.x.

Goloboff, P.A. 1999. Analyzing large data sets in reasonable times: solutions for composite optima. Cladistics, **15**(4): 415–428. doi:10.1111/j.1096-0031.1999.tb00278.x.

Goloboff, P.A. 2013. Extended implied weighting. Cladistics, **30**(3): 260–272. doi:10.1111/cla.12047.

Goloboff, P.A., Carpenter, J.M., Arias, J.S., and Esquivel, D.R.M. 2008*a*. Weighting against homoplasy improves phylogenetic analysis of morphological data sets. Cladistics, **24**(5): 758–773. doi:10.1111/j.1096-0031.2008.00209.x.

Goloboff, P.A., Farris, J.S., and Nixon, K.C. 2008*b*. TNT, a free program for phylogenetic analysis. Cladistics, **24**(5): 774–786. doi:10.1111/j.1096-0031.2008.00217.x.

Greguss, P. 1955. Identification of living gymnosperms on the basis of xylotomy. Akadémiai Kiadó, Budapest. p. 263.

Guindon, S., and Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. **52**(5): 696–704. doi:10.1080/10635150390235520. PMID: 14530136.

Hart, J.A. 1987. A cladistic analysis of conifers: preliminary results. J. Arnold Arbor., Harv. Univ. **68**(3): 269–307.

Hillis, D.M., and Bull, J.J. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. **42**(2): 182–192. doi:10.1093/sysbio/42.2.182.

Hillis, D.M., Huelsenbeck, J.P., and Cunningham, C.W. 1994. Application and accuracy of molecular phylogenies. Science, **264**(5159): 671–677. doi:10.1126/science.8171318.

Holman, G.P. 2014. Phylogenetic systematics in Pinaceae (pine family). Ph.D. thesis, School of Biology and Ecology, University of Maine, Orono, Maine.

Hu, Y.S., and Wang, F.H. 1984. Anatomical studies of *Cathaya* (Pinaceae). Am. J. Bot. **71**(5): 727–735. doi:10.2307/2443370.

Huerta Crespo, J. 1976. Anatomía de la madera de 12 especies de coníferas mexicanas. Boletín Técnico Instituto Nacional de Investigaciones Forestales. p. 51.

Ickert-Bond, S.M. 2000. Cuticle micromorphology of *Pinus krempfii* Lecomte (Pinaceae) and additional species from Southeast Asia. Int. J. Plant Sci. **161**(2): 301–317. doi:10.1086/314243. PMID:10777455.

Jeffrey, E.C. 1905. The comparative anatomy and phylogeny of the Coniferales. Part 2, the Abietineae. Boston Society of Natural History, Boston. p. 37.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, **28**(12): 1647–1649. doi:10.1093/bioinformatics/bts199. PMID:22543367.

Klymiuk, A.A., and Stockey, R.A. 2012. A lower Cretaceous (Valanginian) seed cone provides the earliest fossil record for *Picea* (Pinaceae). Am. J. Bot. **99**(6): 1069–1082. doi:10.3732/ajb.1100568. PMID:22623610.

Klymiuk, A.A., Stockey, R.A., and Rothwell, G.W. 2011. The first organismal concept for an extinct species of Pinaceae: *Pinus arnoldii* Miller. Int. J. Plant Sci. **172**(2): 294–313. doi:10.1086/657649.

Leslie, A.B., Beaulieu, J.M., Rai, H.S., Crane, P.R., Donoghue, M.J., and Mathews, S. 2012. Hemisphere-scale differences in conifer evolutionary dynamics. Proc. Natl. Acad. Sci. U. S. A. **109**(40): 16217–16221. doi:10.1073/pnas.1213621109. PMID:22988083.

Lewis, P.O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. **50**(6): 913–925. doi:10.1080/106351501753462876. PMID:12116640.

Lin, C.-P., Huang, J.-P., Wu, C.-S., Hsu, C.-Y., and Chaw, S.-M. 2010. Comparative chloroplast genomics reveals the evolution of Pinaceae genera and subfamilies. Genome Biol. Evol. **2**: 504–517. doi:10.1093/gbe/evq036. PMID:20651328.

Lin, J.-X., Hu, Y.-S., and Wang, F.-H. 1995. Wood and bark anatomy of *Nothotsuga* (Pinaceae). Ann. Mo. Bot. Gard. **82**(4): 603–609. doi:10.2307/2399841.

Lu, Y., Ran, J.-H., Guo, D.-M., Yang, Z.-Y., and Wang, X.-Q. 2014.

Phylogeny and divergence times of gymnosperms inferred from single-copy nuclear genes. PLoS One, **9**: e107679. doi:10.1371/journal.pone.0107679. PMID:25222863.

Maddison, W.P., and Maddison, D.R. 2010. Mesquite: a modular system for evolutionary analysis, version 2.75 [online]. Available from http://mesquiteproject.org.

Mason-Gamer, R.J., and Kellogg, E.A. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). Syst. Biol. **45**(4): 524–545. doi:10.1093/sysbio/45.4.524.

Mathews, S. 2009. Phylogenetic relationships among seed plants: persistent questions and the limits of molecular data. Am. J. Bot. **96**(1): 228–236. doi:10.3732/ajb.0800178. PMID:21628186.

Mathews, S., Clements, M.D., and Beilstein, M.A. 2010. A duplicate gene rooting of seed plants and the phylogenetic position of flowering plants. Philos. Trans. R. Soc., B, **365**(1539): 383–395. doi:10.1098/rstb.2009.0233.

Miller, C.N., Jr. 1976. Early evolution in the Pinaceae. Rev. Palaeobot. Palynol. **21**(1): 101–117. doi:10.1016/0034-6667(76)90024-5.

Nixon, K.C. 1996. Paleobotany in cladistics and cladistics in paleobotany: enlightenment and uncertainty. Rev. Palaeobot. Palynol. **90**(3–4): 361–373. doi:10.1016/0034-6667(95)00092-5.

Nixon, K.C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics, **15**(4): 407–414. doi:10.1111/j.1096-0031.1999.tb00277.x.

Nixon, K.C., and Davis, J.I. 1991. Polymorphic taxa, missing values and cladistic analysis. Cladistics, **7**(3): 233–241. doi:10.1111/j.1096-0031.1991.tb00036.x.

Nixon, K.C., Crepet, W.L., Stevenson, D., and Friis, E.M. 1994. A reevaluation of seed plant phylogeny. Ann. Mo. Bot. Gard. **81**(3): 484–533. doi:10.2307/2399901.

Ohsawa, T., Nishida, M., and Nishida, H. 1992. Structure and affinities of the petrified plants from the Cretaceous of Northern Japan and Saghalien. XII. *Obirastrobus* gen. nov., petrified pinaceous cones from the Upper Cretaceous of Hokkaido. Bot. Mag., Tokyo, **105**(3): 461–484. doi:10.1007/BF02497661.

Parks, M., Cronn, R., and Liston, A. 2009. Increasing phylogenetic resolution at low taxonomic levels using massively parallel sequencing of chloroplast genomes. BMC Biol. **7**: 84. doi:10.1186/1741-7007-7-84. PMID:19954512.

Parks, M., Cronn, R., and Liston, A. 2012. Separating the wheat from the chaff: mitigating the effects of noise in a plastome phylogenomic data set from *Pinus* L. (Pinaceae). BMC Evol. Biol. **12**: 100. doi:10.1186/1471-2148-12-100. PMID:22731878.

Pol, D., and Escapa, I.H. 2009. Unstable taxa in cladistic analysis: identification and the assessment of relevant characters. Cladistics, **25**(5): 515–527. doi:10.1111/j.1096-0031.2009.00258.x.

Powell, G.R. 2009. Lives of conifers: a comparative account of the coniferous trees. The Johns Hopkins University Press, Baltimore, Md. p. 288.

Price, R.A., Olsen-Stojkovich, J., and Lowenstein, J.M. 1987. Relationships among the genera of Pinaceae: an immunological comparison. Syst. Bot. **12**(1): 91–97. doi:10.2307/2419217.

Pyhäjärvi, T., García-Gil, M.R., Knürr, T., Mikkonen, M., Wachowiak, W., and Savolainen, O. 2007. Demographic history has influenced nucleotide diversity in European *Pinus sylvestris* populations. Genetics, **177**(3): 1713–1724. doi:10.1534/genetics.107.077099. PMID:18039881.

R Core Team. 2014. R: a language and environment for statistical computing. Vienna, Austria. Available from http://www.R-project.org/.

Radais, M. 1894. Contribution à l'anatomie comparée du fruit des conifères. E. Bernard et Cie, Paris, France. p. 172.

Rambaut, A. 2009. FigTree. Tree figure drawing tool v1.4.0. Available from http://tree.bio.ed.ac.uk/software/figtree/.

Rambaut, A., Suchard, M.A., Xie, D., and Drummond, A.J. 2014. Tracer v1.6. Available from http://beast.bio.ed.ac.uk/Tracer.

Richter, H.G., Grosser, D., Heinz, I., and Gasson, P.E. (IAWA Committee). 2004. IAWA list of microscopic features for softwood identification. IAWA Jl, 25: 1–70.

Ronquist, F., Klopfstein, S., Vilhelmsen, L., Schulmeister, S., Murray, D.L., and Rasnitsyn, A.P. 2012*a*. A total-evidence approach to dating with fossils, applied to the early radiation of the Hymenoptera. Syst. Biol. **61**(6): 973–999. doi:10.1093/sysbio/sys058. PMID:22723471.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., et al. 2012*b*. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. **61**(3): 539–542. doi:10.1093/sysbio/sys029. PMID:22357727.

Rothwell, G.W., and Serbet, R. 1994. Lignophyte phylogeny and the evolution of spermatophytes: a numerical cladistic analysis. Syst. Bot. **19**(3): 443–482. doi:10.2307/2419767.

Rothwell, G.W., Mapes, G., Stockey, R.A., and Hilton, J. 2012. The seed cone *Eathiestrobus* gen. nov.: fossil evidence for a Jurassic origin of Pinaceae. Am. J. Bot. **99**(4): 708–720. doi:10.3732/ajb.1100595. PMID:22491001.

Ryberg, P.E., Rothwell, G.W., Stockey, R.A., Hilton, J., Mapes, G., and Riding, J.B. 2012. Reconsidering relationships among stem and crown group Pinaceae: oldest record of the genus *Pinus* from the Early Cretaceous of Yorkshire, United Kingdom. Int. J. Plant Sci. **173**(8): 917–932. doi:10.1086/667228.

Sacher, J.A. 1954. Structure and seasonal activity of the shoot apices of *Pinus lambertiana* and *Pinus ponderosa*. Am. J. Bot. **41**(9): 749–759. doi:10.2307/2438961.

Schmidt, M., and Schneider-Poetsch, H.A.W. 2002. The evolution of gymnosperms redrawn by phytochrome genes: the Gnetatae appear at the base of the gymnosperms. J. Mol. Evol. **54**(6): 715–724. doi:10.1007/s00239-001-0042-9. PMID:12029353.

Sereno, P.C. 2007. Logical basis for morphological characters in phylogenetics. Cladistics, **23**(6): 565–587. doi:10.1111/j.1096-0031.2007.00161.x.

Smith, S.Y., and Stockey, R.A. 2001. A new species of *Pityostrobus* from the Lower Cretaceous of California and its bearing on the evolution of Pinaceae. Int. J. Plant Sci. **162**(3): 669–681. doi:10.1086/320131.

Smith, S.Y., and Stockey, R.A. 2002. Permineralized pine cones from the Cretaceous of Vancouver Island, British Columbia. Int. J. Plant Sci. **163**(1): 185–196. doi:10.1086/324553.

Smith, S.Y., Stockey, R.A., Rothwell, G.W., and Little, S.A. 2016. A new species of *Pityostrobus* (Pinaceae) from the Cretaceous of California: moving towards understanding the Cretaceous radiation of Pinaceae. J. Syst. Paleontol. In press. doi:10.1080/14772019.2016.1143885.

Stockey, R.A. 1977. Reproductive biology of the Cerro Cuadrado (Jurassic) fossil conifers: *Pararaucaria patagonica*. Am. J. Bot. 64(6): 733–734. Retrieved from http://www.jstor.org/stable/2441726. [accessed 1 September 2016].

Swofford, D.L. 2002. PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4. Smithsonian Institution, Washington, USA.

Van Tieghem, P. 1891. Structure et affinités des *Abies* et des genres les plus voisins. Bull. Soc. Bot. Fr. **38**(9): 406–415. doi:10.1080/00378941.1891.10828599.

Wang, X.-Q., Tank, D.C., and Sang, T. 2000. Phylogeny and divergence times in Pinaceae: evidence from three genomes. Mol. Biol. Evol. **17**(5): 773–781. doi:10.1093/oxfordjournals.molbev.a026356. PMID:10779538.

Whang, S.S., Pak, J.-H., Hill, R.S., and Kim, K. 2001. Cuticle micromorphology of leaves of *Pinus* (Pinaceae) from Mexico and Central America. Bot. J. Linn. Soc. **135**(4): 349–373. doi:10.1111/j.1095-8339.2001.tb00787.x.

Whang, S.S., Kim, K., and Hill, R.S. 2004. Cuticle micromorphology of leaves of *Pinus* (Pinaceae) from North America. Bot. J. Linn. Soc. **144**(3): 303–320. doi:10.1111/j.1095-8339.2003.00245.x.

Wiens, J.J. 2006. Missing data and the design of phylogenetic analyses. J. Biomed. Inf. **39**(1): 34–42. doi:10.1016/j.jbi.2005.04.001.

Wiens, J.J., Kuczynski, C.A., Townsend, T., Reeder, T.W., Mulcahy, D.G., and Sites, J.W., Jr. 2010. Combining phylogenomics and fossils in higher-level squamate reptile phylogeny: molecular data change the placement of fossil taxa. Syst. Biol. **59**(6): 674–688. doi:10.1093/sysbio/syq048. PMID:20930035.

Wu, H., and Hu, Z.-H. 1997. Comparative anatomy of resin ducts of the Pinaceae. Trees, **11**(3): 135–143. doi:10.1007/s004680050069.

Appendix A. Characters and their states

Branching and growth

1. Thick bark type when mature: blocks (0); fibrous (1). Scored from personal observations and literature (Eckenwalder 2009).

2. Block bark texture: smooth (0); rough (1). Scored from personal observations and literature (Farjon 1990, 2010).

3. Shoot apex leader: erect (0); spreading (1); drooping (2). Scored from personal observations and literature (Farjon 1990; Eckenwalder 2009; Powell 2009).

4. Short shoots: absent (0); present (1). Only species with extreme dimorphism were scored as having short shoots. Character 2 of Hart (1987). Also similar to character 29 of Farjon (1990). *Cryptomeria*, and *Sciadopitys* are scored as having short shoots, in contrast to scoring by Hart (1987).

5. Short shoot growth: indeterminate (0); determinate (1). The short shoots of *Pinus*, *Cryptomeria*, and *Sciadopitys* are scored as having determinate growth, in contrast to scoring by Hart (1987).

6. Short shoots deciduous with leaves: absent (0); present (1).

7. Lateral branching: orthotropic (0); plagiotropic (1). Scored from personal observations and literature (Farjon 2010).

8. Sex distribution: monoecious (0); dioecious (1). Character 48 of Hart (1987).

Stem and wood anatomy

10. Pith sclerenchyma: absent (0); present (1). Both fibers (elongated cells with tapered ends) and sclereids (stone cells) are included together.

11. Sieve element plastids: starch accumulating (0); protein accumulating (1). Character 4 of Hart (1987). Scored from literature (Behnke 1974).

12. Pith resin canals: absent (0); present (1). Scored from literature (Jeffrey 1905; Sacher 1954).

13. Bars of Sanio: absent (0); present (1). Character 16 of Hart (1987). Scored from literature (Gerry 1910; Chamberlain 1935; Hu and Wang 1984).

14. Axial tracheid tertiary wall helical thickenings: absent (0); present (1); Greguss type (2). Modified from character 13 of Hart (1987). Scored from literature (Jeffrey 1905; Bailey 1909; Greguss 1955).

15. Biseriate bordered pits: absent (0); present (1). Modified from character 12 of Hart (1987). Scored from literature (Greguss 1955).

16. Bordered pit shape: hexagonal (0); circular (1). Modified from character 12 of Hart (1987). Scored from literature (Greguss 1955).

17. Torus: absent (0); present (1).

18. Wood parenchyma axial: absent (0); present (1). Modified from character 9 of Hart (1987), although this did not distinguish between axial and horizontal parenchyma. Scored from literature (Greguss 1955; Esteban and de Palacios 2009).

19. Wood resin canals axial: absent (0); present (1). Modified from character 17 of Hart (1987), which did not distinguish between axial and horizontal canals. Scored from literature (Jeffrey 1905; Greguss 1955).

20. Wood resin canal epithelial cell wall thickness: thin (0); thick (1). Scored from literature (Greguss 1955; Lin et al. 1995).

21. Wood ray series: uniseriate or biseriate (0); some multiseriate (1).

22. Wood ray wall helical thickenings: absent (0); present (1). Modified from character 13 of Hart (1987), which specifies that these are in early wood. Scored from literature (Bailey 1909; Greguss 1955).

23. Wood ray horizontal resin canals: absent (0); present (1). Modified from character 19 of Hart (1987), resin ducts in rays. Scored from literature (Greguss 1955; Baas et al. 1986).

24. Wood ray cross field pitting: small circles (0); fenestriform (1). Modified from character 25 of Hart (1987). Scored from literature (Greguss 1955; Baas et al. 1986; Hart 1987).

25. Wood ray parenchyma horizontal wall indentations: absent (0); present (1). Modified from character 11 of Hart (1987), with states “smooth” versus “nodular or pitted”. Scored from literature (Greguss 1955; Baas et al. 1986).

26. Wood ray tracheids: absent (0); present (1). Character 23 of Hart (1987). Scored from literature (Greguss 1955; Huerta Crespo 1976; Lin et al. 1995; Ickert-Bond 2000; Esteban and Palacios 2009).

27. Wood ray tracheid wall dentation Hudson gradations: one (0); two (1); three (2); four (3); five (4); six (5). Scored from literature (Ickert-Bond 2000; Richter et al. 2004).

28. Stem phloem fibers: absent (0); present (1). Character 6 of Hart (1987). Scored from literature (Jeffrey 1905).

29. Phloem fiber sclereids: absent (0); present (1). Character 7 of Hart (1987). Scored from literature (Jeffrey 1905; Hart 1987).

30. Phloem mucilage: absent (0); present (1). Character 8 of Hart (1987).

31. Phloem resin cavities: absent (0); present (1).

32. Cortical secretory canals: absent (0); present (1). Scored from literature (Jeffrey 1905; Wu and Hu 1997).

Roots

33. Root secretory canals: absent (0); present (1). Scored from literature (Van Tieghem 1891; Jeffrey 1905; Farjon 1990).

34. Root secretory canal number: one (0); two (1); more than two (2). Scored from literature (see previous character).

Leaves

35. Leaf heteroblasty: absent (0); present (1). Heteroblasty is defined as ontogenetic differences in development, as in the presence of primary and secondary leaves in pines.

36. Apical meristem leaves: shorter leaves interrupting growth (0); scale leaves (1); winter buds, tips free (2). Character 37 of Hart (1987).

37. Winter buds resinous: absent (0); present (1). Scored from literature (Farjon 2010).

38. Vegetative cataphylls: absent (0); present (1). This character is only variable in the outgroup.

39. Fascicle sheath around secondary leaves composed of scale leaves: absent (0); present (1).

40. Fascicle sheath retention: deciduous (0); persistent (1). Scored from personal observations.

41. Leaf persistence: perennial (0); annual (1). Character 2 (36) of Hart (1987).

42. Leaf venation: unbranched (0); dichotomous (1). Scored from literature (Chamberlain 1935).

43. Leaf attachment: decurrent (0); stalklike constrictions (sterigma) (1); shield shaped (2). Character 32 of Hart (1987). Scored from personal observations and literature (Farjon 2010).

44. Leaf petiole: absent (0); present (1). Scored from literature (Pant and Basu 1977; Rothwell and Warner 1984).

45. Leaf shape: falcate in profile and tetragonal in cross section (0); linear or lanceolate and bifacially flattened (1); scale-like (2); bilaterally flattened (3); needlelike (4); double or fused (5). Character 28 of Hart (1987); scored from personal observations and literature (Farjon 2010).

46. Needles per fascicle: one (0); two (1); three (2); four (3); five (4). This character varies in pines. Scored from personal observations and literature (Farjon 2005, 2010).

47. Leaf margin: entire (0); serrulate (1). Scored from personal observations and literature (Farjon 1990, 2010; Ickert-Bond 2000).

48. Leaf midrib: raised (0); sunken or absent (1). Scored from personal observations and literature (Pant and Basu 1977; Hart 1987; Farjon 2010).

49. Stomatal orientation: random (0); primarily longitudinal (1); primarily transverse (2).

50. Stomata on upper surface: always absent (0); present (1). Modified from character 38 of Hart (1987). Scored from personal observations and literature (Pant and Basu 1977).

51. Subsidiary cell number: the modal value was used (Eckenwalder 2009; Whang et al. 2001, 2004).

52. Florin rings: absent (0); present (1).

53. Mesophyll parenchyma shape: smooth (0); plicate (1).

54. Mesophyll with palisade parenchyma: absent (0); present (1).

55. Mesophyll secretory canals: absent (0); present (1).

56. Mesophyll resin canal primary position: external (0); medial (1); septal only (2) internal (3).

57. Resin duct number: treated as a continuous character with median and range.

58. Mesophyll with astrosclereids: absent (0); present (1).

59. Endoderm Casparian strips: absent (0); present (1).

60. Vascular bundle number: one (0); two (1); more than two (2).

61. Transfusion tissue tracheid distribution: lateral to vascular bundle (0); all around vascular bundle (1). Character 41 of Hart (1987).

Pollen cones and pollen

62. Pollen cone arrangement: single (0); clusters from a single bud (1); helically arranged (2). Modified from character 51 of Hart (1987).

63. Microsporangia per sporophyll: one (0); two (1); three (2); four to fifteen (3). Modified from character 54 of Hart (1987).

64. Microsporangial position: terminal (0); abaxial (1); adaxial (2). Character 50 of Hart (1987).

65. Microsporangial aggregations: absent (0); present (1).

66. Microsporangial dehiscence: longitudinal (0); oblique (1); transverse (2). Character 55 of Hart (1987).

67. Pollen tetrad formation: simultaneous (tetrahedal) (0); successive (bilateral) (1). Character 57 of Hart (1987).

68. Pollen germination (distal): shallow functional germ furrow (0); harmomegathus (1); functionless germ furrow (2); pore (3). Character 58 of Hart (1987).

69. Pollen sexine: tegillate (0); rough corrugate (1); granular (2); roughened (3). Character 61 of Hart (1987).

70. Pollen annular thickenings: absent (0); present (1). Character 63 of Hart (1987).

71. Pollen triradiate streaks: absent (0); present (1). Character 64 of Hart (1987).

72. Pollen sacci: absent (0); present (1). Similar to character 65 of Hart (1987).

73. Pollen grain prothalial cells: absent (0); present (1). Character 68 (in part) of Hart (1987).

74. Sperm nuclei cell walls: absent (0); present (1). Character 69 of Hart (1987).

Seed cones

75. Seed cone position: terminal (0); lateral (1). Modified from character 98 of Hart (1987).

76. Ovulate strobilus compound: absent (0); present (1). Modified from character 99 of Hart (1987).

77. Compound strobilus as woody cones: absent (0); present (1). Modified from character 104 of Hart (1987).

78. Seed cone orientation at maturity: pendant (0); erect (1). Character 112 of Hart (1987).

79. Cones persistent: absent (0); present (1). Cones are retained on the branch for a long time after seed dehiscence.

80. Cone axis disintegration: absent (0); present (1).

81. Cone method of seed release: cone spreading (0); scale abscission from cone axis (1). Modified from character 111 of Hart (1987). Character 28 of Smith and Stockey (2002).

82. Bract scale complexes: many (0); few (1).

83. Axillary complex: free (0); separating from bract near base (1); partially fused (less than half of the bract length) (2); fused almost to apex (3). Modified from character 101 of Hart (1987).

84. Bract and ovuliferous scale manner of separation: laterally first (0); medially first (1); all at once (2). Modified from character 16 of Smith and Stockey (2002). Taxa scored as with the "do not separate" by Smith and Stockey were scored here as inapplicable.

85. Bract length relative to ovuliferous scale: shorter (0); equal (1); longer (2). Character 2 of Smith and Stockey (2002).

86. Bract base abaxial lobe or cone-scale complex: absent (0); present (1). Similar to character 102 (bract "keeled") of Hart (1987). Character 17 of Smith and Stockey (2002).

87. Bract apex tridentate: absent (0); present (1).

88. Ovuliferous scale or short shoots symmetry: radial (0); somewhat flattened (1); bilateral or scales (2). Modified from character 100 of Hart (1987).

89. Ovuliferous scale at right angles to cone axis for length of seed body with sharply upturned distal portion: absent (0); present (1). Character 27 of Smith and Stockey (2002).

90. Ovuliferous scale shape: flabellate to cuneate (broad at apex) (0); round to rhomboid (broad in middle) (1); tongue-shaped (2); subcordate deltate triangular (3). Fossil cone scales were scored when a surface view was available. Scored from personal observations and literature (Frankis 1988). Scored as inapplicable in species with lateral fertile shoots and Voltzian conifers.

91. Ovuliferous scale base: pedicillate (0); broad (1). Scored from personal observations and literature (Frankis 1988). Radais (1894) used "pedicels" to refer to the fused part of the bract scale complex. We scored scales as pedicillate when the base was less than 4 mm wide for 4 mm or more.

92. Ovuliferous scale apex distinct lobes: absent (0); present (1). Ryberg et al. (2012).

93. Seeds embedded in a pocket of scale tissue. Cheirolepidiaceae (*Pararaucaria*) bears seeds this way, interseminal ridge is absent.

94. Interseminal ridge: absent (0); present (1). Character 26 (in part) of Smith and Stockey (2002).

95. Interseminal ridge extension: extending less than half of seed diameter (0); extending more than half of seed diameter (1); extending between and overarching (2). Character 26 (in part) of Smith and Stockey (2002).

96. Ovuliferous scale or bract-scale complex trichomes: absent (0); present (1). Character 11 from Smith and Stockey (2002) is "trichomes on the cone axis, scale, or bract base". We have restricted the character to the scale or bract-scale complex to narrow the character concept and facilitate scoring.

97. Ovuliferous scale apex: thinning distally (0); thickening distally into an apophysis (1). Character 1 of Smith and Stockey (2002).

98. Umbo: absent (0); present (1).

99. Umbo position: dorsal (0); terminal (1).

100. Spine: absent (0); present (1).

101. Cortical resin canals: absent (0); present (1).

102. Cortical resin canal diameter: uniform (0); dilated markedly near points of branching (1). Character 10 of Smith and Stockey (2002). We used a 5:1 criterion for scoring resin canals as markedly dilated.

103. Inner cortex sclerenchyma: absent (0); present (1). Character 8 of Smith and Stockey (2002).

104. Outer cortex sclerenchyma: absent (0); present (1). Character 9 of Smith and Stockey (2002). Hypodermis (fibers) should not be scored as present, rather only sclereids.

105. Secondary vascular tissue continuity: forming a continuous cylinder or little dissected (0); in separate strands (1). Character 5 of Smith and Stockey (2002).

106. Secondary xylem continuity: forming a continuous cylinder or little dissected (0); in separate strands (1).

107. Secondary xylem growth increments: one (0); two (1). Character 7 of Smith and Stockey (2002).

108. Secondary xylem resin canals: absent (0); present (1). Character 6 of Smith and Stockey (2002).

109. Bract and scale trace origin: separate (0); united (1). Character 12 of Smith and Stockey (2002).

110. Scale trace derivation: clearly from two lateral strands (0); single abaxially concave strand (1). Character 13 of Smith and Stockey (2002). Taxa with traces that are derived from two lateral strands, but fuse to form an abaxially concave strand (e.g., *Obirastrobus*, *Pseudoaraucaria*, and some species of *Pityostrobus*) were scored as 1 (see Smith and Stockey 2002).

111. Scale trace shape at level of inner cortex: abaxially concave (0); flat, becoming cylindrical after divergence (1). Character 14 of Smith and Stockey (2002).

112. Resin canals to cone scale complex arising from cortical canals: as a single branch (0); two origins (1); three separate origins (2); four separate origins (3); more than four separate origins (4). Character 15 of Smith and Stockey (2002). The total number of branches originating from cortical resin canals from both sides of the scale trace.

113. Pith resin canals: absent (0); present (1). Character 4 of Smith and Stockey (2002).

114. Pith sclerenchyma: absent (0); present (1). Character 3 of Smith and Stockey (2002). Both fibers (elongated

cells with tapered ends) and sclereids (stone cells) are included without distinction.

115. Bract number of vascular bundles: one (0); more than one (1). Determined from a cross section of the bract.

116. Bract trace extension: entering bract (0); terminating before entering free part of bract (1). Character 20 of Smith and Stockey (2002).

117. Bract trace vascular ray: absent (0); present (1). Character 21 of Smith and Stockey (2002).

118. Bract resin canals: absent (0); present (1). Character 19 (in part) of Smith and Stockey (2002).

119. Bract resin canal number: two (0); more than two (1); one (2). Character 19 (in part) of Smith and Stockey (2002).

120. Bract sclerenchyma: absent (0); present (1). Character 18 of Smith and Stockey (2002).

121. Ovuliferous scale vascular bundles distal to seed shape: straight (0); adaxially convex (1).

122. Ovuliferous scale resin canals: absent (0); present (1).

123. Ovuliferous scale trace with resin canal inside: absent (0); present (1).

124. Resin canals abaxial to ovuliferous scales at scale base: absent (0); present (1). Character 22 (in part) of Smith and Stockey (2002).

125. Resin canals adaxial to ovuliferous scales at scale base: absent (0); present (1). Character 22 (in part) of Smith and Stockey (2002).

126. Mechanical tissue of scale base: highly gelatinous, modified (0); well developed (1). Character 14 from Alvin (1988).

127. Mechanical tissue of scale base composition: highly gelatinous (0); fibrous (1). Character 15 from Alvin (1988).

128. Sclerenchyma in ovuliferous scale abaxial to vascular tissue: absent (0); present (1). Character 25 (in part) of Smith and Stockey (2002).

129. Sclerenchyma in ovuliferous scale adaxial to vascular tissue: absent (0); present (1). Character 25 (in part) of Smith and Stockey (2002).

130. Sclerotic nests or clusters in distal part of scale: absent (0); present (1).

Ovules and seeds

131. Pollination drop: absent (0); present (1). Character 71 of Hart (1987).

132. Micropyle symmetry: symmetric (0); asymmetric (1). Character 73 of Hart (1987).

133. Ventral canal cell: distinct cell wall (0); no wall but nuclei (1). Character 74 of Hart (1987).

134. Megaspore membrane thickness: thick double (0); thin (1). Character 77 of Hart (1987).

135. Megaspore membrane thickness at micropylar end: uniform thickness (0); thin at micropylar end (1). Character 78 of Hart (1987).

136. Proembryo wall formation: secondary type (0); primary type (1). Character 87 of Hart (1987).

137. Proembryo tiers: three (0); four (1). Character 89 of Hart (1987).

138. Polyembryony: simple (0); cleavage (1); Character 97 of Hart (1987).

139. Ovule position: adaxial (0); terminal (1); lateral (2); from all sides (3).

140. Ovule attachment: appendicular on sporophyll or equivalent (0); on reduced shoot (1).

141. Ovule orientation: erect (0); inverted (1). Character 114 of Hart (1987).

142. Ovules number per ovuliferous scale: one (0); two (1); three or more (2). Character 115 of Hart (1987). Character 33 of Smith and Stockey (2002).

143. Seed wing insertion: adnate (0); articulate (1); not attached (2).

144. Resin vesicles or cavities in integument: absent (0); present (1). Character 30 of Smith and Stockey (2002).

145. Sclerotestal thickness: thin (0); thick (1).

146. Ridged sclerotesta: absent (0); present (1). Character 31 of Smith and Stockey (2002).

147. Enlarged parenchyma pad or cushion at chalazal end of seed: absent (0); present (1). Character 32 of Smith and Stockey (2002).

148. Seed body shape: ovoid or obovate (0); triangular or cuneate or oblong (1); cordate (2).

149. Vascular strand entering seed: absent (0); present (1).

150. Seed wings formed from ovuliferous scale tissue: absent (0); present (1). Character 29 (in part) of Smith and Stockey (2002).

151. Seed wings formed from sarcotestal tissue: absent (0); present (1). Character 29 (in part) of Smith and Stockey (2002).

152. Seed wing attachment: deep cup that folds around sides (0); shallow cup (1); claws (2); covering entire seed (3).

153. Type of seed germination: hypogeal (0); epigeal (1).

154. Cotyledon number: two (0); 3 to 7, sometimes 8 (1); 8 to 11, as few as six (2); 12 or more (3). Modified from character 121 of Hart (1987).

Chemistry

155. Resins: absent (0); present (0).

156. Seed storage product: starch (0); oils (1). Character 116 of Hart (1987).

157. Biflavonoids: absent (0); present (1). Character 43 of Hart (1987).

158. Tropolones: absent (0); present (1). Character 46 of Hart (1987).

References

Alvin, K.L. 1988. On a new specimen of *Pseudoaraucaria major* Fliche (Pinaceae) from the Cretaceous of the Isle of Wight. Bot. J. Linn. Soc. **97**(2): 159–170. doi:10.1111/j.1095-8339.1988.tb02458.x.

Baas, P., Schmid, R., and Van Heuven, B.J. 1986. Wood anatomy of *Pinus longaeva* (bristlecone pine) and the sustained length-on-age increase of its tracheids. IAWA Bull. **7**(3): 221–228. doi:10.1163/22941932-90000988.

Bailey, I.W. 1909. The structure of the wood in the Pineae. Bot. Gaz. **48**(1): 47–55. Available from http://www.jstor.org/stable/2467584 [accessed 20 February 2016]. doi:10.1086/329935.

Behnke, H.-D. 1974. Sieve-element plastids of Gymnospermae: their ultrastructure in relation to systematics. Plant Syst. Evol. **123**(1): 1–12. doi:10.1007/BF00983281.

Chamberlain, C.J. 1935. Gymnosperms structure and evolution. Dover Publications, Inc., New York.

Eckenwalder, J.E. 2009. Conifers of the world: the complete reference. Timber Press, Portland, Ore.

Esteban, L.G., and de Palacios, P. 2009. Comparative wood anatomy in Abietoideae (Pinaceae). Bot. J. Linn Soc. **160**(2): 184–196. doi:10.1111/j.1095-8339.2009.00971.x.

Farjon, A. 1990. Pinaceae: drawings and descriptions of the genera *Abies*, *Cedrus*, *Pseudolarix*, *Keteleeria*, *Nothotsuga*, *Tsuga*, *Cathaya*, *Pseudotsuga*, *Larix* and *Picea*. Regnum Veg. **121**: 1–330.

Farjon, A. 2010. A handbook of the world's conifers. Brill, Leiden, Netherlands.

Frankis, M. 1988. Generic inter-relationships in Pinaceae. Notes R. Bot. Gard. Edinb. **45**: 527–548.

Gerry, E. 1910. The distribution of the 'bars of Sanio' in the Coniferales. Ann. Bot. **24**(1): 119–123. Available from http://aob.oxfordjournals.org/content/os-24/1/119.extract [accessed 20 February 2016].

Greguss, P. 1955. Identification of living gymnosperms on the basis of xylotomy. Akadémiai Kiadó, Budapest.

Hart, J.A. 1987. A cladistic analysis of conifers: preliminary results. J. Arnold Arbor., Harv. Univ. **68**(3): 269–307.

Hu, Y.S., and Wang, F.H. 1984. Anatomical studies of *Cathaya* (Pinaceae). Am. J. Bot. **71**(5): 727–735. Available from http://www.jstor.org/stable/2443370 [accessed 20 February 2016]. doi:10.2307/2443370.

Huerta Crespo, J. 1976. Anatomía de la madera de 12 especies de coníferas mexicanas. Boletín Técnico Instituto Nacional de Investigaciones Forestales, **8**: 1–51.

Ickert-Bond, S.M. 2000. Cuticle micromorphology of *Pinus krempfii* Lecomte (Pinaceae) and additional species from Southeast Asia. Int. J. Plant Sci. **161**(2): 301–317. doi:10.1086/314243. PMID:10777455.

Jeffrey, E.C. 1905. The comparative anatomy and phylogeny of the Coniferales. Part 2. The Abietineae. Mem. Boston Soc. Nat. Hist. **6**: 1–37.

Lin, J.-X., Hu, Y.-S., and Wang, F.-H. 1995. Wood and bark anatomy of *Nothotsuga* (Pinaceae). Ann. Mo. Bot. Gard. **82**(4): 603–609. doi:10.2307/2399841.

Pant, D.D., and Basu, N. 1977. A comparative study of the leaves of *Cathaya argyrophylla* Chun & Kuang and three species of *Keteleeria* Carriere. Bot. J. Linn. Soc. **75**: 271–282. doi:10.1111/j.1095-8339.1977.tb01488.x.

Powell, G.R. 2009. Lives of conifers: a comparative account of the coniferous trees. The Johns Hopkins University Press, Baltimore, Md.

Radais, M. 1894. Contribution à l'anatomie comparée du fruit des conifères. Ann. Sci. Nat. Bot. Biol. Veg. **7**: 165–368.

Rothwell, G.W., and Warner, S. 1984. *Cordaixylon dumusum* n.sp. (Cordaitales). I. Vegetative Structures. Bot. Gaz. **145**: 275–291. Available from http://www.jstor.org/stable/2474349 [accessed 10 August 2016].

Ryberg, P.E., Rothwell, G.W., Stockey, R.A., Hilton, J., Mapes, G., and Riding, J.B. 2012. Reconsidering relationships among stem and crown group Pinaceae: oldest record of the genus *Pinus* from the Early Cretaceous of Yorkshire, United Kingdom. Int. J. Plant Sci. **173**(8): 917–932. doi:10.1086/667228.

Sacher, J.A. 1954. Structure and seasonal activity of the shoot apices of *Pinus lambertiana* and *Pinus ponderosa*. Am. J. Bot. **41**(9): 749–759. Available from http://www.jstor.org/stable/2438961 [accessed 20 February 2016]. doi:10.2307/2438961.

Smith, S.Y., and Stockey, R.A. 2002. Permineralized pine cones from the Cretaceous of Vancouver Island, British Columbia. Int. J. Plant Sci. **163**(1): 185–196. doi:10.1086/324553.

Van Tieghem, P. 1891. Structure et affinités des *Abies* et des genres les plus voisins. Bull. Soc. Bot. Fr. **38**(9): 406–415. doi:10.1080/00378941.1891.10828599.

Whang, S.S., and Pak, J.-H. 2001. Cuticle micromorphology of leaves of *Pinus* (Pinaceae) from Mexico and Central America. Bot. J. Linn. Soc. **135**(4): 349–373. doi:10.1111/j.1095-8339.2001.tb00787.x.

Whang, S.S., Kim, K., and Hill, R.S. 2004. Cuticle micromorphology of leaves of *Pinus* (Pinaceae) from North America. Bot. J. Linn. Soc. **144**(3): 303–320. doi:10.1111/j.1095-8339.2003.00245.x.

Wu, H., and Hu, Z.-H. 1997. Comparative anatomy of resin ducts in Pinaceae. Trees, **11**(3): 135–143. doi:10.1007/s004680050069.